The potential of sorghum as a feedstock source for bioethanol production in Nigeria

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In partial fulfilment of the requirement for the award of Doctor of Philosophy (PhD) Degree

By

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Sept., 2013

Declaration

I, Muhammad Nasidi, hereby consciously declare that the following thesis is based on the results of investigations wholly conducted by myself, and that it is of my own composition. This thesis has not, in whole or in part, been previously presented for a higher degree or qualification. All works used in the course of this work are clearly referenced as appropriate.

ABSTRACT

This thesis investigated the potential of sorghum as a feedstock source for bioethanol production in Nigeria. Sorghum is a cereal with high tolerance for varied environmental and climatic stresses. It can produce starch-rich grains, sweet stalk juice and high lignocellulosic biomass, depending on the crop variety and cultivation location. Nigeria is the third largest sorghum producer worldwide, but less than 10% of sorghum produced has commercial applications. For example, the grains represent a staple food source or can be utilised as a brewing adjunct. The stalk juices are used in syrup production while the green field residues (bagasse) are partly used in forage production and fencing but mostly left in field for burning. This thesis has shown that sorghum crops have alternative uses in liquid biofuel production.

In this study, SSV2, KSV8 and KSV3 sorghum cultivars were cultivated under rain fed conditions without chemical fertilizers in Kano and Kaduna, Nigeria. The climate in Kano is relatively warmer and drier than Kaduna, with Kano favouring higher biomass yields and Kaduna favouring higher sugary stalk juice yield. Total dry bagasse yields in Kano were 29 t/ha, 33 t/ha and 37 t/ha for SSV2, KSV8 and KSV3 crops, respectively. For crops harvested in Kaduna, the yields were 24 t/ha and 31 t/ha for SSV2 and KSV8, respectively. Furthermore, raw stalk juice yields of 25000 L/ha, 23300 L/ha and 22600 L/ha were obtained for SSV2, KSV8 and KSV3 in Kano and 25500 L/ha and 24500 L/ha for SSV2 and KSV8 in Kaduna. Total fermentable sugar (analysed by HPLC) in Kano-grown SSV2, KSV8 and KSV3 sorghum juices were 144 g/L, 66 g/L and 104 g/L, respectively, compared with 162 g/L and 88 g/L for SSV2 and KSV8 juices from Kaduna-grown sorghum. Fermentations of different sorghum juices were performed with *Saccharomyces cerevisiae* (without exogenous nutrient supplementation) and produced ethanol yields (measured by GC-MS) of 65 g/L, 36 g/L and 62 g/L for SSV2, KSV8 and KSV3 juices in Kano while Kaduna juice fermentations produced 81 g/L and 52 g/L ethanol for SSV2 and KSV8, respectively. Supplementation of sorghum juices with additional nutrients improved fermentation performance. Floured husked grains from different sorghum cultivars were separately mashed with a combination of various enzyme cocktails, followed by fermentations of the mashes with *S. cerevisiae*. Ethanol yields of 355 L/t, 421 L/t and 379 L/t were obtained for SSV2, KSV8 and KSV3, respectively, and this fermentation performance was also verified by CO_2 gas evolution as observed by the ANKOM^{RF} gas monitoring system. Another yeast, *Pichia stipitis* showed lower corresponding ethanol yields when fermenting sorghum grain mashes. Experiments were also conducted to convert sorghum lignocellulose residues (bagasse) to ethanol. Pre-treatment of the bagasse fractions followed by detoxification of the enzymatic hydrolysates with calcium hydroxide over-liming and charcoal filtration showed ethanol yields of 23 g/L and 20 g/L for SSV2 and KSV3 (Kano) on fermentation with *Pachysolen tannophilus* (without nutrient supplementation) while *S.cerevisiae* yielded corresponding ethanol of 21 g/L and 19 g/L respectively Results from this research have shown that whilst sorghum cultivar SSV2 is a very favourable feedstock for bioconversion to ethanol from juice in Kaduna and bagasse in Kano, the KSV8 cultivar is better suited when exploiting husked grain starch as source for bioethanol production in Nigeria.

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DEDICATION

This is for my beloved wife, Murjanatu Isa Kaita and my wonderful boys; Zayd, Ahmad & Anas.

I love you all, dearest.

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CHAPTER ONE

Literature review

1.0 Introduction.

Greenhouse gas emissions from continued use of fossil based fuels are considered precusor to global warming (Aleklett, 2012). Therefore, climate change concern together with increase uncertainty in future prices of fossil fuels necessitated the search for alternative and renewable energy sources (Olsson and Ahring 2007; Shiva, 2009). Fossil fuels are reported to account for over 80% of primary energy source globally, of which about 58% is expended as transport fuel (Nigam and Singh, 2011). In not too distant future, biofuels comprising bioethanol, biobutanol and biodiesel are envisaged as likely alternatives to fossil fuels in the transport sector, this is largely because of their renewability and sustainability (Dennis *et al.,* 2008). Bioethanol is strategically important as a transport fuel of the future. It is an environment friendly energy source which generates acceptable quality exhaust gases, leading to reduced greenhouse gas (GHG) emissions (Chiras, 2009; Nigam and Singh, 2011). Bioethanol, a plant-based liquid biofuel, may be used in automobiles either as an additive or substitute to petroleum as transport fuel (Pandey, 2009). Plant biomass such as grains, sugary stalk juices and lignocellulosic materials are potential feedstock sources for bioethanol production (Brennan and Owende, 2010).

Nigeria, being the 9th largest oil producing country in the world largely depends on fossil fuels as cheap sources of energy. This has in the past stunted desired growth

in the renewable energy sector of the country (Abila, 2010; Ishola *et al.,* 2013). However, in recent times, the Nigerian government in partnership with the private sector has significantly intensified efforts towards diversification of the country's energy supply mix. This is in part through massive investment in developing a vibrant renewable energy sector in the country (Vincent-Akpu, 2012).

1.1 Bioethanol, the alternative transport fuel

At the moment, bioethanol remains the most promising renewable energy for the 21st century transport industry (Defra, 2006). Among properties that make bioethanol attractive for use as transport fuel is its compatibility with gasoline (Table 1.1). It can be used as an additive or substitute for gasoline in automobiles with no engine modification requirement for up to 10% bioethanol and 90% gasoline blend. However, higher bioethanol blends such as E-85 can only be used in flexible fuel vehicles i.e. FFVs (Rutz and Janssen 2011). Furthermore, among the environmental benefits of bioethanol use as a transport fuel are its biodegradability and low toxicity to the environment. It can also replace lead in gasoline as an octane rating enhancer. Furthermore, bioethanol causes little or no environmental pollution during combustion, because carbon dioxide and water are the major combustion products of bioethanol. Because E-5 to E-10 fuel blends (i.e. 5-10% bioethanol and 95-90% gasoline) may be used with little or no modifications in conventional vehicle engines, several countries around the world have formulated deliberate policies to encourage use of this fuel blend range (Macedo *et al.,* 2008; Pandey 2009). In Nigeria, the federal government, through its 2007 National Biofuel Policy statement aspires to fully implement use of E-10 fuel blend as transport fuel in all gasoline-driven vehicles nation wide by 2020 (Nasidi *et al.,* 2010).

Table 1.1 Bioethanol physico-chemical properties

Parameter	**Value**
Molecular formula	C_2H_5OH
Molecular mass	46.07 g/mol
Appearance	Colourless liquid
Density	0.789 kg/L
Boiling temperature	78.5^{o}C (173^{o}F)
Freezing point	-117^{o}C
Flash point	12.8^{o}C
Ignition temperature	425^{o}C
Explosion limits:	
-lower	3.5% v/v
-upper	19% v/v
Vapour pressure @ 38^{o}C	50 mmHG
Heating values @ 20^{o}C:	
-higher heating value	29800 kJ/kg
-lower heating value	21090 kJ/kg
Acidity (pKa)	15.9
Viscosity @ 20^{o}C	1.200 mPa.s
Refractive index (η_D) @ 25^{o}C	1.36
Octane number rating	99

Source: Walker (2010).

The Nigerian transport sector comprising mainly of rail, road and air thrives more or less exclusively on fossil derived fuels. The transport sector's demand for fossil fuels represents over 67% of the total fossil fuel consumed in the country (Dayo, 2008). In addition to the transport sector's bioethanol demand, over 3.5 billion litres of bioethanol is also required annually to meet chemical industries and domestic cooking gel production demands (Azih, 2007; Iwayem *et. al.,* 2010). However, over 90% of bioethanol currently consumed in Nigeria is imported while the combined domestic production output is less than 134 million litres per annum (Ohimain, 2010; Ishola *et al.,* 2013). Therefore, there is wide supply-demand deficit in the Nigerian bioethanol domestic market.

1.2 Bioethanol production overview

In planning for bio-fuel production, identification of feedstock sources with long-term sustainability must be put into consideration. The sustainability of a feedstock source would normally depend largely on the production environment and other uses of the potential feedstock chosen (Vries *et al.,* 2010). For example, in spite of the preference of starch-based feedstocks for bioethanol production, their use as a primary staple food source for billions of people triggered a lot of concerns related to food security consideration for the ever growing world population (Serna-Saldivar, 2012). For example, assume a situation where there wasn't a food-fuel debate in the USA, considering the hundred thousands of tons of maize utilised in bioethanol production suggests maize alone cannot support the projected objectives of renewable fuel legislation in that country. The USA has a target of 36 billion gallons of bioethanol demand established for 2022. Hence, to meet such demand, about 333 million tons of maize would be required to be dedicated for bioconversion to ethanol annually. This represents about twice the current total annual maize production output of China, which happens to be among the top five maize producers in the world (Wortmann and Regassa, 2011). Consequently, this suggested maize would not be available for any other use in USA except for bioethanol production and even at that, the land requirement to meet such demand would interfere with land availability for food crop production. Therefore, the use of food related feedstocks for bioethanol production may be considered not sustainable.

At the moment, commercially viable production processes for bioethanol predominantly rely on 1st generation feedstocks such as starch, cane molasses, juices from sugarcane, sweet sorghum or sugar beets (Walker, 2010). This is mainly because starch and juices contain easily accessible fermentable sugars that may be

liberated by simple pre-treatment and hydrolysis processes. These are crucial considerations for viable economic production processes. However, concerns on food security issues as previously discussed, in addition to the costs of starch based feedstocks considerations necessitates the research and development of alternative 2nd generation bioethanol feedstocks (Fig. 1.1). The 2nd generation feedstocks are cellulosic-based (lignocellulosic biomass) which makes them less competitive with food supply chain and in addition to having the benefits of low commercial value. The 3rd generation feedstocks which comprise seaweeds, algae and so on are still at the preliminary research stage (Drapcho et al., 2008).

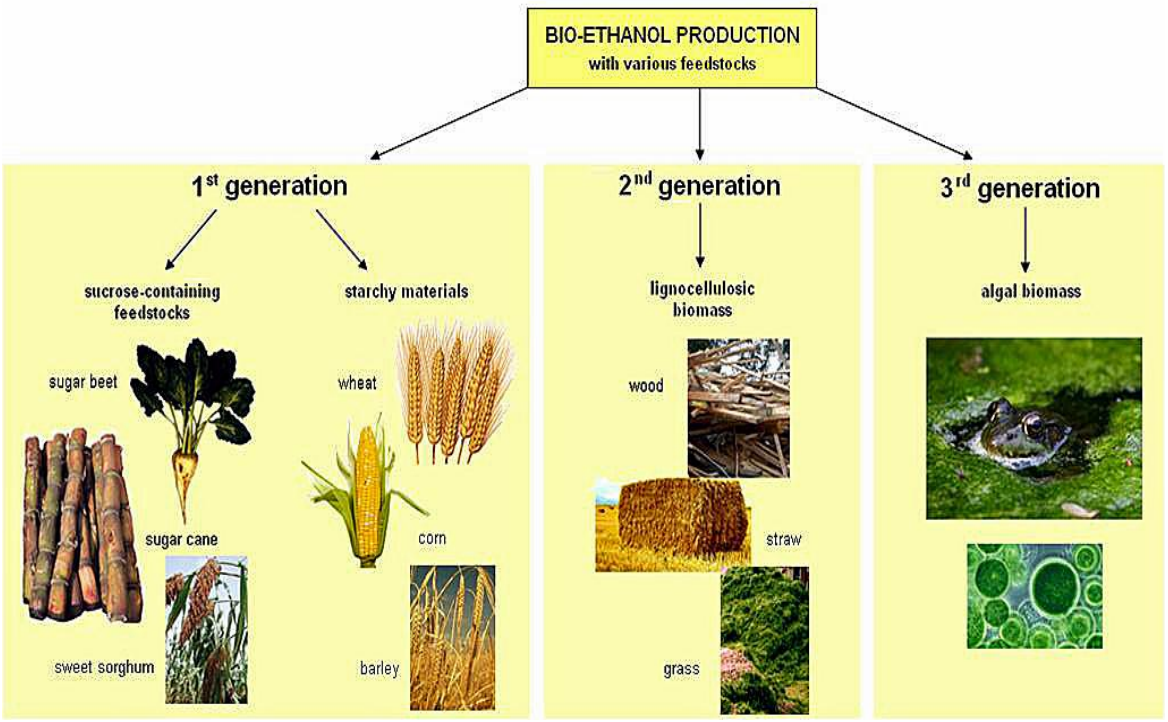

Fig. 1.1 Examples of Bioethanol feedstocks. In 1st generation feedstocks, the sucrose-containing substrate may be directly converted to ethanol. However, the starchy-based substrates must be hydrolysed to liberate fermentable sugars. For the 2nd generation feedstock, the lignocellulose substrates will be de-lignified and the cellulose-hemicellulose materials dgraded to liberate sugars. Study on 3rd generation feedstocks is currently ongoing. Source: www.googleimages.com

According to the Global Renewable Fuels Alliance report (GRFA, 2012), the future outlook of global ethanol industry continues to look promising, particularly with

regards to rapid development of new technologies that are based on utilisation of 2nd generation feedstocks for commercial scale feasible bioethanol production. This is in addition to the continued re-engineering of fermentation yeasts to make them more robust and versatile in their fermentative capacity (Boulton and Quain, 2001; Jimoh *et al.,* 2011). The global bioethanol sector is not only important because it is a vehicle for reduced GHG emissions, but because it also has a social significance. For example, it supported over 1.4 million jobs worldwide and contributed an estimated $277.3 billion to the global economy in 2010. Furthermore, bioethanol is foreseen to continue contributing towards the displacement of hundreds of millions of fossil fuels barrels annually, thereby reducing the burden of crude oil importation costs in non-oil producing countries (FAOSAT, 2012). Data for progressive global bioethanol production by regions are summarised in Table 1.2. The USA and Brazil followed by China remain the top three leading bioethanol producing nations worldwide (Ogundari *et al.,* 2012). It was observed that the African content has the least rate of growth in its bioethanol sector. However, it is pertinent to observe that despite the relative low growth, the production output appeared to be progressively on the increase over the years (Table 1.2).

Table 1.2 World bioethanol Production output (Million Litres)

Region	**2006**	**2007**	**2008**	**2009**	**2010**	**2011**	**2012**
Europe	1627	1882	2855	3645	4254	4429	4973
Africa	0	55	65	100	130	150	235
North America	18716	25271	35946	42141	51584	54765	54580
South America	16969	20275	24456	24275	25964	21637	21335
Asia	1940	2142	2753	2927	3115	3520	3965
World total	**39252**	**49625**	**66075**	**73088**	**85047**	**84501**	**85088**

Source: GRFA (2012).

In Nigeria, commercial scale bioethanol production commenced since 1972 and yet the country depended on importation to meet over 90% of its domestic demand for

bioethanol consumption. For example, over 123 million litres of bioethanol was imported in 2007 by the Nigerian National Petroleum Corporation (NNPC) to meet domestic demand for E-10 fuel blending (Nasidi *et al.,* 2010). Although over the past five years several bioethanol production projects have been initiated (Table 1.3), the total combined installed plants output of 134 million litres per annum will merely represent about 10% of the estimated annual 1.3 billion litres of ethanol requirement for the transport sector alone. This is not to even mention the annual 3.75 billion litres of bioethanol demand for domestic cooking ethanol gel demand (Ohimain, 2010). However, several other projects were further initiated by the Federal government of Nigeria through NNPC (Nigerian National Petroleum Corporation) and in partnership with the private sector (Table 1.4). It is envisaged that on completion and commissioning of these projects, the bioethanol demand-supply gap would be significantly bridged to a reasonable extent. Ohimain (2013) estimated that about US$3.86 billion has been invested into the construction of these nineteen integrated biorefining plants (Table 1.4) and the combined annual production output of these plants is projected at about 2.66 billion litres of fuel alcohol. Furthermore, fourteen additional new bioethanol mini plant projects are projected to be in the offing soon (Agboola *et al.,* 2011; Galadima *et al.,* 2011). The Nigerian government has shown demonstrated commitment towards the advancement of its bioethanol agenda of replacing 10% of total national petroleum consumption with bioethanol by 2020. However, some keen industry watchers argued that the failure of the country to locally meet 25% of the total 10 percent bioethanol demand for E-10 fuel blending by 2010 is an indication that the ambitious policy of attaining E-10 fuel use in all petrol driven vehicles nation wide by 2020 may not be feasible within the set timeline (Ogundari *et al*., 2012).

Table 1.3 Bioethanol production output in Nigeria

Company	Plant location	Feedstock	Installed capacity (million L/yr)
Alconi/Nosak	Lagos	Imported crude ethanol	43.8
UNIKEM	Lagos	Imported crude ethanol	65.7
Intercontinental distilleries	Ota-Idiroko	Imported crude ethanol	9.1
Dura clean	Bacita	Molasses/cassava	4.4
AADL	Sango-Ota	Cassava	10.9
Total			**133.9**

Source: Ohimain (2010).

Table 1.4 Summary of bioethanol production projects initiated in Nigeria

Project type	Estimated cost (USD, $)	Promoters	Feedstock	Feedstock demand (t/yr)	Project output (ethanol/yr)	Total land requirement (ha)	Project phase
Integrated biorefinery & sugar mill	306 million	[a]NNPC/JVC	Sugarcane	1.8 million	75 million litres, 116810 metric tons refined sugar, 59MW electricity.	20000 ha of which16000 ha will be cultivated.	In progress
Integrated biorefinery & sugar mill	306 million	NNPC/JVC	Sugarcane	1.8 million	75 million litres, 116810 metric tons refined sugar, 59MW electricity.	20000 ha of which16000 ha will be cultivated.	In progress
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Fuel alcohol	80-100 million	NNPC/JVC	Sugarcane	1.8 million	120 million litres, 10-15 MW electricity.	26374 ha land to be cultivated.	Planning stage
Fuel alcohol	125 million	NNPC/JVC	cassava	3-4 million	40-60 million litres.	15000 ha of land.	EPIC[b]
Fuel alcohol	125 million	NNPC/JVC	cassava	3-4 million	40-60 million litres.	15000 ha of land.	EPIC[b]
Integrated biorefinery & sorghum farm	70 million	GBL	Sorghum stalk juice	1.05 million litres (est.)	84 million litres + grains.	30000 ha acquired.	EPIC[b]
Integrated biorefinery & sorghum farm	92 million	GBL	Sorghum stalk juice	385000 litres (est.)	30.8 million litres + grains.	11000 ha acquired.	EPIC[b]
Integrated biorefinery & sugar mill	300 million	Ethanig	Sugarcane	3.25 million	100 million litres + refined sugar + electricity.	50000 ha.	Planning stage
Integrated biorefinery & sugar mill	300 million	Ethanig	Sugarcane	3.25 million	100 million litres + refined sugar + electricity.	50000 ha.	Designing stage
Integrated biorefinery & sugar mill	167 million	Savannah sugar Co.	Sugarcane	1.0 million	100 million litres, refine sugar, 300MW electricity.	20000-36000 ha.	Planning stage
Fuel alcohol	90 million	Casplex ltd	Cassava	300,000	38.86 million litres.	15000ha.	EPIC[b]
Fuel alcohol	18 million	Oke-Ayedu	Cassava	238,500	38.1 million litres.	15000ha.	EPIC[b]
Fuel alcohol & starch	122 million	CrowNet	Cassava	150,000	65 million litres + 100000 tons starch and 50 000 tCO_2/day.	12500ha.	Commissioned
Fuel alcohol	115 million	Taraba	Cassava	300000	72 million litres + 360 000 tons starch + 1.87 million tCO_2 +57Mgy liquid fertilizer + 1600MW electr.	30000ha.	EPIC[b]
Cooking ethanol gel	1 billion	Nig. Govt.	cassava	8 million	1.44 billion litres gel.	400000ha.	EPIC

Source: Agboola *et al*. (2011) and Ohimain, (2013). [a]**NNPC/JVC:** Nigerian National Petroleum Corporation/Joint Venture Coys. [b]**EPIC:** Engineering, Procurement, Installation and Commissioning (Turnkey project).

1.3 Bioethanol feedstocks

Bioethanol is conventionally produced from plant-based biomass by extracting fermentable sugars for subsequent fermentation with yeasts (Walker, 2011). Plant biomass broadly comprised grains, stalk juices and lignocelluloses (Nigam and Singh, 2011). However, assessing sustainability of growing feedstock for bioethanol production will include consideration of the efficient use of land, water, energy resources and agrochemicals among others (Vries *et al.,* 2010). There is no doubt that diversion of resources meant for food crops production to energy crops cultivation will undermine food production capacity of the country thereby exacerbating hunger on the general populace. Hence, the attempt by the Nigerian government's biofuel programme to directly use food related crops such as cassava, sorghum grains and/or juice as well as sugarcane juice as feedstock sources for bioethanol production is considered unsustainable and will impact negatively on the country's quest for attaining sustainable food security targets (Baiyegunhi and Fraser, 2009; Olembo *et al.,* 2010). For example, while sorghum grains are in no doubt an important staple food source in Nigeria, the sugar mill factories in Nigeria depend on imported sugarcane raw juices to meet over 50% demand of their feedstock for refined sugar production. Therefore, further diversion of the locally cultivated limited sugarcane crops for bioethanol production will further aggravate the situation. Furthermore, the clearing and dedication of forests reserves and woodlands for energy crops cultivation will not augur well for the country's desire to achieve 25% forest coverage in compliance with international best land management practice standards (Chavan *et al.,* 2009; Abila, 2010).

It is pertinent to mention that while sugarcane and sweet sorghum stalk juices contain readily fermentable sugars such as sucrose and glucose that may be directly

fermented by yeasts to bioethanol (Almodares and Hadi, 2009), the grains (starch feedstock) contain carbohydrates that need to be enzymatically hydrolysed to liberate fermentable sugars such as maltose and glucose that are fermentable to bioethanol by yeasts (Agu *et al.,* 2006; Ijasan *et al.,* 2011). However, lignocellulosic biomass comprises complex mixture of celluloses and hemicelluloses polysaccharides that are bound by tough lignin materials (Cao *et al.,* 2012). Therefore, certain pre-treatment steps are required to liberate fermentable sugars from the celluloses and hemicelluloses polysaccharides for fermentation. It is the additional pre-treatment method for lignocellulose biomass that poses challenges for commercial scale utilisation of these feedstocks (Chandel *et al*., 2011). Figures 1.2 and 1.4 give an overview of production processes of bioethanol from first and second generation feedstocks respectively.

1.3.1 First-generation bioethanol feedstocks

First-generation bioethanol feedstocks are either starch derived biomass (e.g. grains and cassava) or sucrose derived biomass (e.g. sugarcane and sorghum stalk juices). They are economically viable for commercial scale bioethanol production technologies. This is because of the ease in liberation of fermentable sugars from the substrates, particularly juices that may be directly fermented with little or no pre-treatment requirement (Drapcho *et al.,* 2008). However, although the first-generation feedstocks are considered favourable in terms of production technology economics, the food-fuel supply conflict previously discussed raises a lot of debate relating to food security concerns and the sustainability question of these feedstocks (Gouveia, 2011). An overview of 1st generation bioethanol production process is shown in Fig. 1.2 while equations 1.1 and 1.2 represents starch hydrolysis and fermentation processes.

a. **Sucrose derived feedstock:** extracted sugarcane or sweet sorghum stalk juices are clarified, filtered and sterilised. These processes may be followed by juice pH adjustment, nutrient supplementation and enzymatic hydrolysis. The final process is fermentation of the pre-treated juice by preferred yeasts (Andrzejewski *et al.,* 2013).

b. **Starch derived feedstock:** typically, floured grains or cassava are cooked/ mashed with or without exogenous hydrolytic enzymes supplementation under favourable conditions. This is then followed by fermentation of the hydrolysates/mash with favourable yeasts (Prag, 2012).

Starch saccharification stoichiometric equation;

$$\underset{\text{(Starch)}}{(C_6H_{10}O_5)_n} + nH_2O \xrightarrow{\textbf{Amylases}} \underset{\text{(Glucose)}}{n(C_6H_{12}O_6)} \quad \textbf{eqn. 1.1}$$

Fermentation stoichiometric equation;

$$\underset{\text{(Glucose)}}{C_6H_{12}O_5} \xrightarrow{\textbf{Yeasts}} \underset{\text{(Ethanol)}}{2C_2H_5OH} + 2CO_2 \quad \textbf{eqn. 1.2}$$

While juice fermentations may be represented with only equation 1.2, starch feedstocks would have to be represented firstly by equation 1.1 (hydrolysis to release sugars) and then equation 1.2 to represent fermentation of the released sugars.

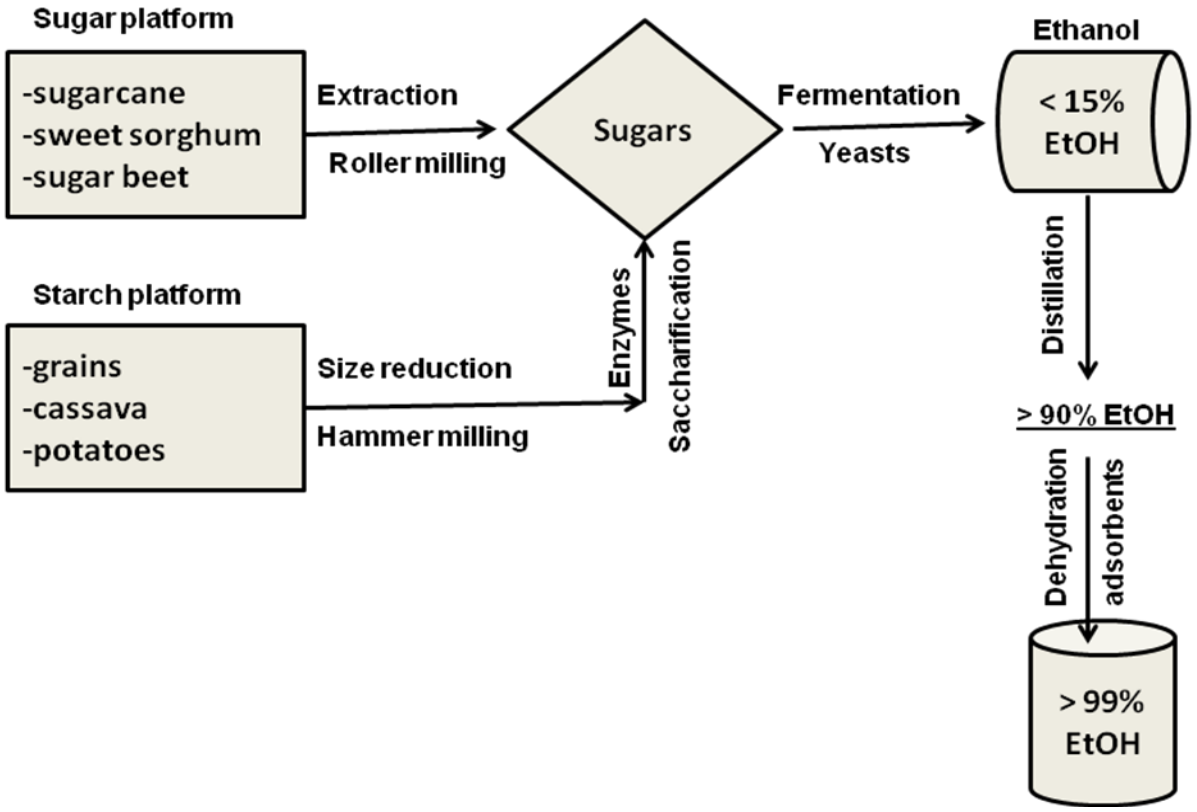

Fig. 1.2. First-generation bioethanol production processes. Brief processes overview for sucrose and starch-based feedstocks bioconversion to ethanol. While sucrose-based feedstocks are bioconverted via sugar platform, the starch-based feedstocks are bioconverted to ethanol via starch platform.

1.3.2 Second-generation bioethanol feedstocks

Lignocellulosic biomass is derived from agricultural residues, trees, forest residues, grasses and other biowaste materials. They are a relatively cheap renewable and sustainable feedstock for bioconversion to ethanol (Chandel *et al.,* 2011). However, there are major challenges in commercial scale utilisation of these feedstocks in bioethanol production. Some of it relates to finding an economically feasible method to overcome the cost of lignocellulosic pre-treatment methods required to liberate the fermentable sugars (Fig. 1.3). The lignocellulosic pre-treatment methods are ideally designed to specifically facilitate disintegration of cellulose, hemicellulose and lignin structure. Consequently, the cellulose and hemicellulose carbohydrates are exposed to enzymatic biodegradation to release monomeric and oligomeric sugars that are

fermentable to yeast (Harmsen *et al*., 2010). Celluloses are essentially crystalline long chain polymers of glucose (hexose sugars) while hemicellulose polymers predominantly consist of xylose and arabinose (pentose sugars). The lignin fraction consists of phenolic building blocks that for all practical purpose and intents are not fermentable by yeasts but may be recovered and utilised as fuel for firing boilers and/or providing process heat and electricity for the ethanol production facility (Chandel *et al.,* 2011; Chen *et al*., 2012). Various lignocellulosic biomass pre-treatment methods such as chemical applications, steam explosion, microwaving, and ultrasound application among others have been previously reported (Gao *et al.,* 2011). However, among all these reported processes, lignocellulose acid hydrolysis methods have continued to attract more commercial application interest, simply because of their cost-effectiveness (Chandel *et al*., 2011). These methods (acid hydrolysis) principally involve extracting sugars from lignocellulose biomass by either concentrated or dilute acid hydrolysis followed by enzymatic hydrolysis and detoxification of the hydrolysates for efficient fermentation processes (Harmsen *et al*., 2010). The generic overview of lignocellulosic bioethanol production process is shown in Fig. 1.4 while equation 1.3 represents the hydrolysis of cellulose polymers to glucose.

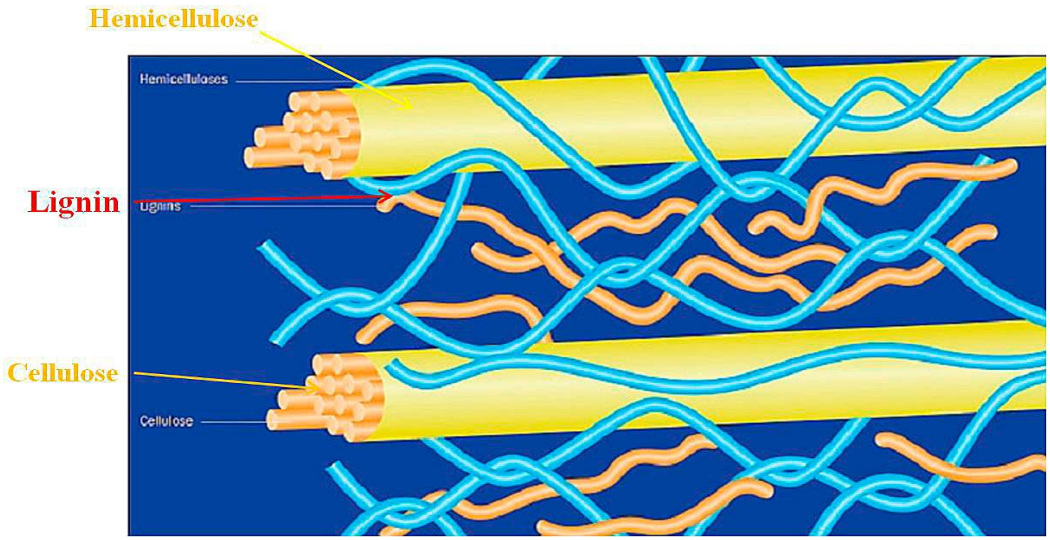

Fig. 1.3 Lignocellulose biomass structure. While lignin fibres are non-fermentable by yeasts, the cellulose and hemicellulose fractions are degraded to simple yeast fermentable sugars. Source: Chandel *et al*. (2011)

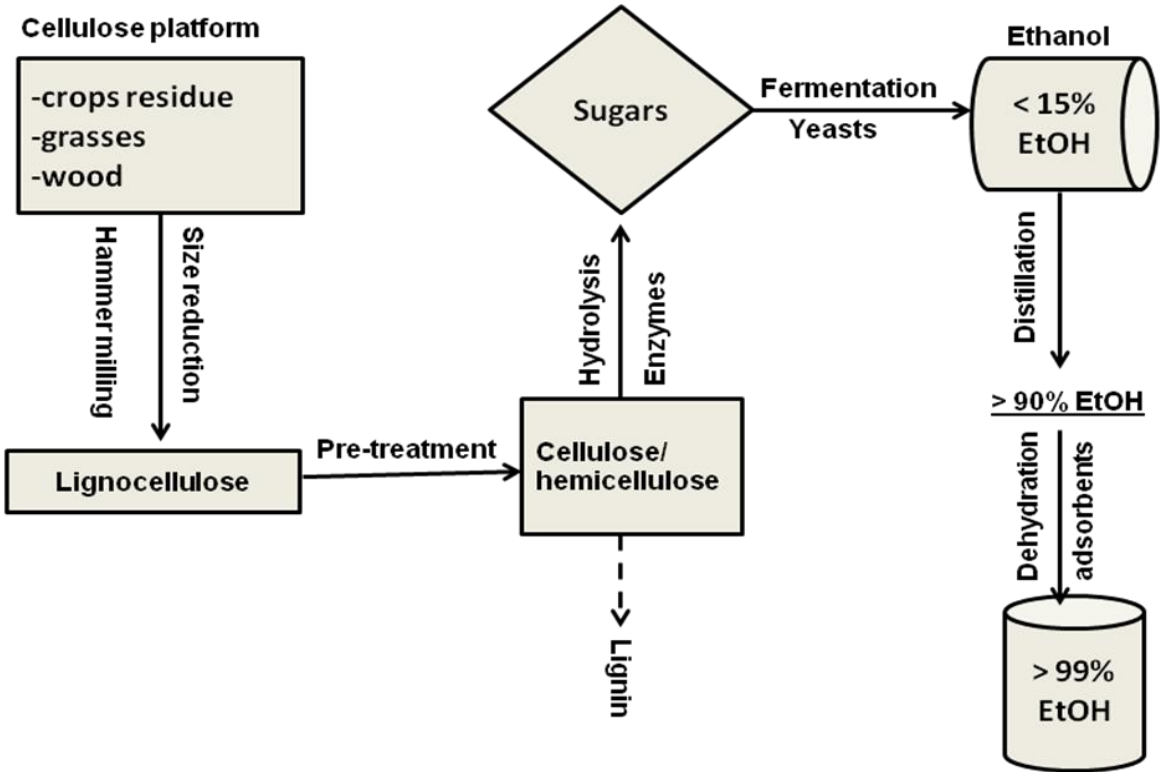

Fig. 1.4 Second-generation bioethanol production processes. Brief processes overview of lignocellulosic-based feedstocks bioconversion to ethanol. The lignocellulose biomass is delignified and the cellulose/hemicellulose substrates are enzymatically hydrolysed to liberate fermentable sugars.

Cellulose material degradation stoichiometric equation:

$$(C_6H_{10}O_5)_n + nH_2O \xrightarrow{\text{Cellulase}} n(C_6H_{12}O_6) \quad \text{eqn. 1.3}$$

(Cellulose) (Glucose)

As previously discussed, food security concerns regarding use of grains, tubers and cane juices for bioconversion to ethanol favours the use of non-food related lignocellulosic materials (in the form of agricultural and forestry residues). Lignocellulose materials are inexpensive and abundant as sustainable feedstocks for bioethanol production (Donghai *et al.,* 2010). Over ten billion metric tons of lignocellulose biomass is produced annually worldwide (Alvira *et al*., 2010), of which Nigeria contributes over 83 million metric tons with an estimated 11 million metric tons being agricultural wastes (Afolayan *et al.,* 2012). For example, in Nigeria, sorghum crop residues alone generate 2-3 million metric tons of lignocellulose biomass waste annually. Less than 40% of the sorghum wastes in Nigeria is utilised as livestock feed and for fence thatching in rural areas while over 60% of the produce is left in fields for burning (PROMISO, 2008; Ismaila *et al.,* 2010). Consequently, these huge sorghum residues may be considered a potentially viable feedstock source for bioethanol production in Nigeria. The sorghum residues compared most favourable to cassava, sugarcane and grains which are the conventional feedstock sources currently being utilised in the country (NNPC, 2007). Furthermore, Nigeria is ranked among the top three sorghum producing countries in the world and therefore, this could be a reasonable indicator that sorghum residues will continue to be abundantly available for utilisation in bioethanol production locally in Nigeria (Nasidi *et al.*, 2010).

1.4 Fermentation microbes (yeasts)

The fermentation performance of ethanol substrates are significantly influenced by the characteristics and efficiency of the fermenting yeasts (Boulton and Quain, 2001). In simple terms, fermentation refers to bioconversion of sugars (e.g. glucose, fructose and sucrose) to cellular energy under anaerobic conditions by yeasts thereby producing alcohol, acids and carbon dioxide as by- or co-products. Typically, wines, beers and other alcoholic beverages are fermentation products (Harmon, 2012). Yeasts are eukaryotic microorganisms belonging to the fungal kingdom. There are over 1500 known species of yeasts (Kurtzman and Fell, 2006). Among common yeasts used in industrial scale applications, the baker's yeasts (*Saccharomyces cerevisiae*) are the most widely used yeasts in brewing and industrial fermentations (Boulton and Quain, 2001). *S. cerevisiae* yeast strains are known to be tolerant of relatively high osmotic pressure (due to high glucose levels in media). The yeasts also show tolerance to high ethanol concentration levels during fermentation and hence they adapt well in fermentation media (Walker, 1998). However, most *S. cerevisiae* strains are only able to efficiently ferment hexose sugars such as glucose but are unable in most cases to ferment pentose sugars e.g. xylose and arabinose (van Maris *et al.,* 2007; Yasuda *et al.,* 2013). Certain yeasts such as *Pachysolen tannophilus* and *Pichia stipitis* among others are known as xylose fermenting yeasts (Liu *et al.,* 2012; Wan *et al*., 2012). Some examples of *S. cerevisae* yeasts strains identified and classified based on their origins in Nigeria are shown in Table 1.5. Various fermentation techniques such as very High gravity (VHG) fermentation, immobilised yeasts fermentations, exogenous nitrogen source supplementations etc have been employed to enhanced yeast fermentation performance (el Mansi and Bryce, 1999; Fayemi and Ojokoh, 2012).

Table 1.5 Examples of *S. cerevisiae* yeast strains and their uses

Strains	Origin	Location/special quality	*Accession no.
NPA30, NPA31, NPA33	Palm wine sample 3		AM900396
NPA041	Palm wine sample 4	Aba, Abia State 2002, Nigeria.	
NPA5a1, NPA5b1, NPA5d1, NPA5d2,	Palm wine sample 5		
NPA61, NPA62	Palm wine sample 6		
NPA71, NPA72	Palm wine sample 7		
NPA81	Palm wine sample 8		
NPAB21, NPAB22,	Palm wine sample nB2	Isiala Ngwa, Abia State, 2004, Nigeria.	
NPAB33, NPAB314	Palm wine sample B3		
NPCR27,	Palm wine sample C2	Uyo, Cross River State, 2004, Nigeria.	AM900394
NPCR414, NPCR415	Palm wine sample C4		
NPCR51	Palm wine sample C5		
NPDR234	Palm wine sample D2	Ikwuano/Oboro, Abia State, 2004, Nigeria	AM900395
NPDR47	Palm wine sample D4		
CBS8856, CBS8857, CBS8858, CBS8859	Sorghum beer	Ghana	AM900399
MUCL28071	Banana wine	Burundi	AM900403
MUCL27815/CBS400	Palm wine	Ivory Coast	AM900397
MUCL30909	Fermented cassava	Burundi	AM900400, AM900401, AM900402
DBVPG1853	White Tecc	Ethiopia	
DBVPG6044	Bili wine	West Africa	
NCYC110	Ginger beer from *Z. officinale*	West Africa	

Source; Ezeronye and Legras (2009). *Accession no. represents strain genetic coding.

1.5 Nigeria: a brief geographical overview

Nigeria, a West African country with an estimated total land area of 923,770 square kilometres and a population of over 160 million people is blessed with over 36 billion

barrels of proven crude oil reserves and 187 cubic feet of natural gas reserves respectively (as at third quarter of 2009). Therefore, Nigeria is ranked the 9th largest oil exporting country worldwide (Abila, 2010; Vincent-Akpu, 2012). However, in spite of the country's massive oil and gas reserves in addition to having oil production output of about 2.1 million barrels of oil daily, Nigeria remains a net refined petroleum products importer (Ohimain, 2013). Nigeria is largely a tropical climate region that is characterized by relatively high temperatures and intense heat particularly in the north. Diurnal temperatures range from a maximum of 45^{o}C in the far north to 31^{o}C in the south, mean rainfall range from 3800 mm at the coastal areas to below 650 mm at the northern region (Agboola *et al.,* 2011; Ishola *et al.,* 2013). Table 1.6 show an overview of Nigeria's land mass distribution and uses.

Table 1.6 Breakdown of land usage in Nigeria (2008)

Land distribution/use	Area (Million ha)	Percentage (%)
Water body (e.g. inland rivers/lakes)	13.0	14.1
Arable cropland	28.2	30.5
Permanent cropland	2.5	2.7
Pasture land	28.3	30.6
Forest & woodland	10.9	11.8
Fadama (irrigated land)	2.0	2.2
Others	7.5	0.5
Total area	**92.4**	**100.0**

Source: FMARD (2008).

Agriculture used to be the mainstay of the Nigerian economy during the 70's and early 80's. However, the massive jump in oil prices during the 90's led to Nigeria's "economic boom" from oil revenue with consequent negligence of the agricultural sector by successive governments (Ismaila *et al.,* 2010). Typically, agricultural practice in Nigeria is driven by peasant farmers that are scattered across the country and with each farmer holding about 0.5-3.0 hectare of farmland. The peasant farming practice is most commonly characterized by rudimentary farm systems and

low capitalization, invariably resulting in low produce yield per hectare of land cultivated (Kolawale and Ojo, 2007). However, currently, the Nigerian government is committed to revamping the agricultural sector back to its "glorious days" through its recently articulated "National Agricultural Transformation Agenda" scheme. For example, sorghum crop is the second most important cereal (after rice) which the scheme targeted to boost its production. The government targeted to improve the national sorghum production capacity by over 20% in 2011 output i.e. sorghum output is projected to reach about 11.5 million metric tons output by 2014 in Nigeria. Beside the introduction of improved sorghum cultivar seedlings to farmers along with training on modern mechanised farming skills, the sorghum value chain distribution network is also targeted for expansion through creation of more commercial utilisation opportunities for sorghum produce (FMARD, 2012). Table 1.7 shows details of the top five sorghum producing countries.

Table 1.7 Top 5 world sorghum producing countries (2008/2009)

Country	Production (million tons)	Cultivation (million ha)	(%) World production	Productivity (tons/ha)
USA	11.997	2.942	17.51	4.077
Nigeria	9.318	7.617	13.60	1.220
India	7.926	7.764	11.57	1.021
Mexico	6.641	1.833	9.69	3.622
Sudan	3.869	6.619	5.64	0.584

Source: FAOSTAT (2011) and Codex (2012)

A total of about seven million hectares of land is currently dedicated to sorghum cultivation across Nigeria. The savannah region is the hub of sorghum production and accounts for over 85% of the total national sorghum production output (Adejuwon, 2005; Ismaila *et al.,* 2010). Table 1.8 shows sorghum productivity varies across the states of the federation with the productivity increasing from the colder regions to warmer regions; Fig. 1.5 shows Nigerian sorghum crop zones. Industrial

utilisation capacity of sorghum produce remains low in Nigeria. Only about one hundred and twenty thousand metric tonnes of sorghum grains are utilised annually in the Nigerian brewing and beverage sector while by far a lesser quantity is used in the confectionary sector (USAID, 2009). However, demand for white grain sorghum is gradually increasing for malting and brewing purposes since the federal government dramatically increased tariffs on barley importation. Barley grains used to be the sole feedstock for the Nigerian brewing and malting sector. Consequent to the government decision to increase tariff on barley importation, the sector has been forced to adopt white grain sorghum as adjunct in brewing processes and sometimes as the major feedstock in some brewing processes (Ukwuru, 2010; FMARD, 2012). Table 1.9 shows some selected Nigerian sorghum cultivars and their potential industrial applications and agronomic characteristics.

Table 1.8 Nigerian sorghum output in 2007-2008 seasons grown under rain fed

	Land area ('000 ha)		Grain output ('000 Mt)		Productivity (t/ha)	
State	***2007***	***2008***	***2007***	***2008***	***2007***	***2008***
Borno	765.50	773.16	837.05	853.77	1.093	1.104
Yobe	176.78	180.16	172.65	176.65	0.977	0.981
Bauchi	250.23	257.74	272.91	292.01	1.091	0.883
Gombe	139.82	141.22	141.17	142.58	1.010	1.010
Adamawa	142.50	142.77	164.32	164.65	1.153	1.153
Jigawa	204.94	209.58	119.62	123.63	0.584	0.590
Katsina	339.59	346.38	343.11	376.39	1.010	1.087
Sokoto	166.59	176.59	91.24	96.71	0.548	0.548
Kebbi	198.58	206.46	209.66	224.34	1.056	1.087
Zamfara	306.62	330.83	455.21	491.63	1.485	1.486
Kano	320.84	343.75	760.32	813.54	2.370	2.367
Kaduna	330.55	333.86	420.27	424.47	1.271	1.271
Taraba	170.40	176.00	170.40	171.25	1.000	0.973
Plateau	111.83	121.56	116.52	120.05	1.042	0.988
Nasarawa	71.50	73.01	109.22	111.40	1.528	1.526
FCT	29.70	29.85	48.89	49.18	1.646	1.648
Niger	513.52	523.79	612.63	624.88	1.193	1.193
Kwara	67.30	72.69	102.97	116.30	1.530	1.600
Kogi	79.34	84.89	89.99	84.49	1.134	0.995
Benue	110.25	110.74	192.94	192.69	1.750	1.740
TOTAL	**4,496.38**	**4,635.03**	**5,431.09**	**5,650.61**	**1.208**	**1.219**

Source: FMRD (2012).

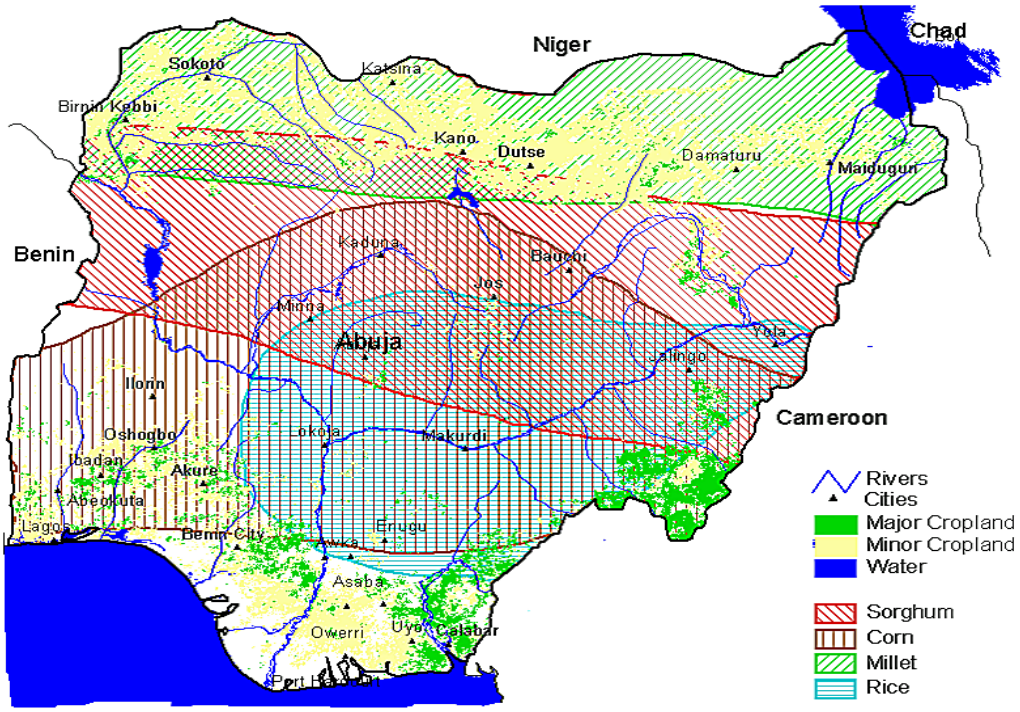

Fig. 1.5 Nigerian crop zones profile. Map of Federal republic of Nigeria showing vegetative zones across regions. Source: www.googleimage.com

Table 1.9 Some selected Nigerian sorghum cultivars and their uses

Cultivar	Origin/pedigree	Basic agronomic features
SS10, NRL-1 SS10, NRL-2 SS10, NRL-3 SS10, NRL-5	Cross bred of pure line derived from single cross L187 x L1499. And developed as L533 at IAR Samaru Zaria, Kaduna.	Semi-dwarf, medium sized loose head with dropping branches; large cream coloured seed. Adapted to Northern and Southern Guinea Savanna. Medium maturing (150-165 days) with potential yield of 1800 – 3000 kg/ha. Good for brewing, striga tolerant, high yielding and good palatability.
SSV 98001 SSV 98002	Developed at IAR Samaru Zaria as collection from local germplasm.	Tall varieties (200 cm – 250 cm) adapted to Sudan and Sahel Savanna. Early maturing (90 – 100 days) and has potential yield of 1500 – 2500 kg/ha.
SK 5912 (Samsorg 17)	Selection from local collection of Kaura through mutation breeding at IAR Samaru.	Semi-dwarf, semi-compact elliptic ear head; medium sized yellow seeds. Adapted to Northern and Southern Guinea Savanna. Late maturing (160-180days) and height of about 140-150cm. Tolerant to striga and resistant to major disease. High yielding and good for brewing.
SSV-8 (Samsorg 22)	Selection developed at IAR Samaru as Line L-181	Semi-dwarf, open-ear head, medium sized seeds adapted to Northern Guinea Savanna and is late maturing (160-180 days) with plant height of 140-150cm.
SSV-9 (Samsorg 23)	Selection from the cross L181 x RZ1 and developed as line 243 at IAR Samaru	Semi-dwarf, elliptical cream coloured seeds. Glume substending seeds, tan coloured and conspicuous. Late maturing (160-180days) adapted to Northern Guinea Savanna. Plant height ranging from 140-150cm and potential yield of 1800-3000kg/ha. Tolerant to striga and resistance to major disease.
KSV11 (SAMSORG-5)		Short season, maturity period 95-105days. Tolerant to striga. Dwarf type, Seed colour white. Potential yield, 1.5-2.5t/ha
KSV8 (SAMSORG14)		Medium season. Maturity period 130-140days. Potential yield 2.5 - 3.0t/ha. Seed colour white
SSV2(FBL) (SAMSORG16)		Potential yield 2.5-3.5t/ha. Seed colour white. Use by industries especially for brewing.

Source: Alhassan and Adedayo (2010); Ismaila *et al.* (2010)

1.6 Sorghum agronomy.

Sorghum bicolor (L) Moench is the most primitive cultivated sorghum race and it is characterized by open inflorescences and long clasping glumes which enclose the usually small grains at maturity as shown in Fig. 1.6. These cultivars are normally grown in Africa and Asia either for grain production (as food or feed) and for their sweet stalk juice that can be utilised for molasses or syrup production (Chopra 2001; Brink and Belay, 2006). These sorghum varieties with sweet stems are most commonly known as sweet sorghums and are closely related to grain sorghum but typically differs from grain sorghum in that the sweet sorghum variety have relatively lower grain yield and have thick tall stalks that accumulates high level of sugars such as sucrose, glucose and fructose (Guiying *et al.,* 2004). Generally, the economic superiority of sweet sorghum over grain sorghum may include its high stalk yield (which constitutes about 70% of fresh biomass yield), extractible sweet juice containing both reducing and non-reducing sugars as well as grain yield. The stalk weight is normally related to the crop height, thickness and juiciness (Audilakshmi *et al.,* 2010). For example, Figs. 1.7 and 1.8 shows SSV2 and KSV3 sorghums at site B (Kano); it is worth noting that KSV3 appeared much taller than SSV2 cultivar.

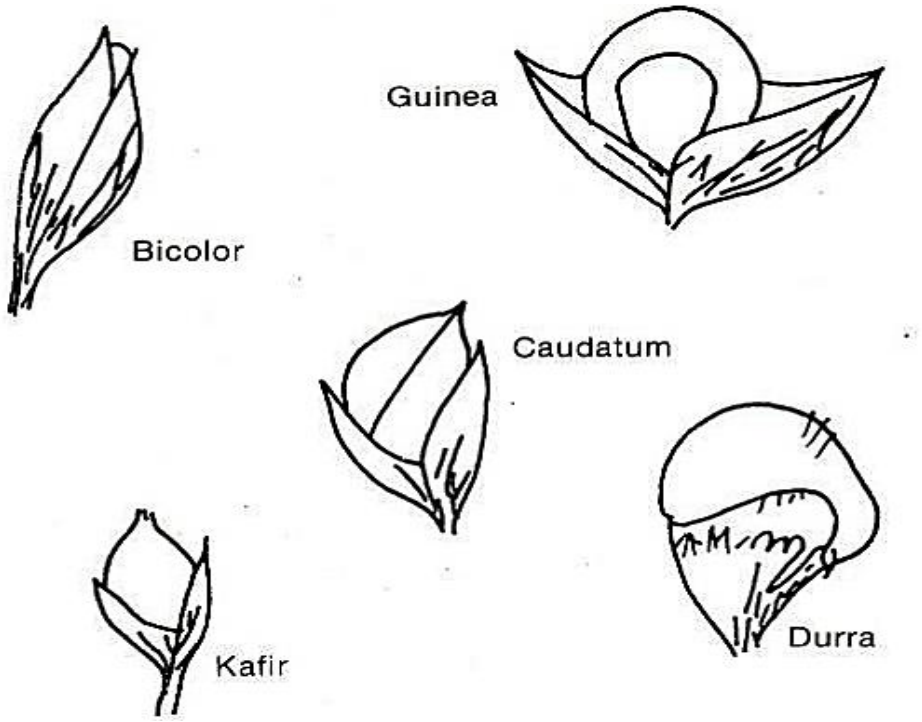

Fig. 1.6 Sorghum land race seed features. Sorghums are broadly classified into five land races characterised partly by seed features. *Sorghum bicolour (L.) Moench* belong to the bicolor land race. Source: Chopra (2001).

Fig. 1.7 SSV2 sorghum gown in Kano. SSV2 sorghum grown in Kano (site B) for the purpose of this study. Crops are shown as at 11 weeks after planting date.

Fig. 1.8 KSV3 sorghum in Kano. KSV3 sorghum grown in Kano (site B) for the purpose of this study. Crops are shown at 16 weeks after planting date.

Previous studies conducted to assess the genetic relationship between sweet sorghum and grain sorghum identified 95 genotypes comprising basic 31 sweet sorghums and 64 grain sorghum species, this constituted the *sorghum bicolor* sub-species. Although many hybrid cultivars between the grain and sweet sorghum genomes are being developed for higher grain and sugar juice yield (Ritter *et al.,* 2007). In general, both sorghum crops variety are considered to have relatively high carbon assimilation rates at 50 g/m^2/day, leading to fast growth rates and efficient rates of CO_2 use (Serna-Saldìvar *et. al.*, 2012). Hence, sorghum crop growth cycles are normally within 3 to 5 months depending on cultivar type and cultivation conditions. Thus, 2 to 3 crop cycles annually is potentially feasible for most sorghum cultivars (Haile and Hofsvang, 2001; Dillon *et al.,* 2007). Although sorghum is more of a dry land crop (Almodares *et al.,* 2010), sufficient soil moisture availability for

plant growth is crucial for improved plant yields. While grain sorghum cultivars will thrive under limited water supply of less than 300 mm rainfall for a season of 100 days, its grain yield will be most favourable with additional rainfall. However, sweet sorghum cultivars will need between 500 to 1000 mm of rainfall to achieve good total above ground biomass yields of 50 to 100 t/ha i.e. on fresh weight basis (Rao *et al.,* 2010). Sorghum's relative water use efficiency appeared more favourable than that of corn, for example, while sorghum requires only 310 kg water to produce 1 kg dry matter, corn will require about 370 kg of water. Furthermore, previous studies showed the annual evapotranspiration rate per annum of sorghum crop to be 580 mm while that of corn is about 760 mm (Worthmann *et al.,* 2011). Among the most important advantage of sorghum over other cereals is its ability to become dormant (particularly at vegetative growth phase) under harsh climatic or environmental conditions and then resume growth when favourable conditions return. For example, under early season-drought conditions, sorghum crop stops growth before panicle initiation and remains vegetative; the crop resumes leaf production and flowering when conditions again become favourable for growth such as improved rainfall or irrigation. However, mid-season drought stops leaf development (Rao *et al,* 2010). Finally, sorghum can be typically grown under 15 to 37^{o}C temperature range, although the optimum growth temperature for efficient photosynthesis is 32 to 34^{o}C under optimum rainfall of 550 to 800 mm and relative humidity of 15 to 50% (Chopra, 2001).

1.6.1 Sorghum crop diseases

The productivity of sorghum crops depends largely on several parameters among which are pest attacks and microbial disease infections controls. For example, sorghum grain mould infection (a condition that usually occurred between the crop's

anthesis and physiological maturity stage) is a serious cause of harvest loss to farmers in Africa (Pande, *et. al.*, 2003; Bandyopadhyay, *et. al.*, 2008). Ezeaku and Gupta (2004) reported that a 2001 survey found an estimated 21 million hectares of land out of the total land area dedicated for sorghum cultivation in Africa was infested with *Striga hermonthica* (a parasitic weed). This resulted in an estimated annual grain loss of about 4.1 million tons and represented a revenue loss of about 7 billion US dollars to farmers. Furthermore, in Africa, *Striga hermonthica* was identified as the most destructive parasitic weed in the western part of the continent and accounting for regional grain losses of 15–25% annually. Some hybrid cultivars cultivated in Nigeria have been identified to be resistant to *Striga* attack and they include KSV4, ICSV111, ICSV400, S-35 and Gaya early (Ezeaku and Gupta, 2004; Agbede and Ojeniyi, 2009; Ogbonna, 2009). Consequently, despite adaptability of sorghum to stressful environmental conditions, pest and fungi attack prevention and control during crop cultivation is necessary (Ritter, *et. al.,* 2007; Almodares, *et. al.*, 2009), these would particularly help prevent or minimise the chances of disease infestation caused by *Striga hermonthica*, aphids, midge, stem borer etc (Ajayi, 1999; Haile and Hofsvang, 2001; de Milliano, 2008; Wortmann, *et. al.*, 2011). USAID (2009) reported that the most common diseases associated with sorghum crops cultivation in Nigeria may be classified as:

a. Diseases that affect germinating seedlings by causing retardation of seedling germination growth rate or death.

b. Diseases that invade leaves causing reduced biomass yield potential and photosynthesis efficiency.

c. And lastly, diseases that attack roots and stems causing root and stem rot, poor stalk juice yield and subsequently low or no grain yield.

Nevertheless, good cultivation management practices could effectively help reduce or eliminate disease attack incidence during sorghum cultivation. For example, proper seedling treatment prior to planting may reduce chances of seed rot and improve germination. Crop rotation and inter-cropping may also reduce the possibility of spreading previous season disease to newly cultivating crops (Ahmad, *et. al*., 2007; Egbe, 2010; Oseni, 2010). Various sorghum crop cultivation management practices aimed at production of sorghum biomass with improved nutrient value for healthy food and livestock feed have been published (Cao, *et. al.*, 2012; Chen, *et. al*., 2012; Han, *et. al.*, 2012; Yu, *et. al.*, 2012). Despite the investigated sorghum crop's high adaptation to adverse climatic conditions, high productivity output remains constrained by poor soil quality, low and erratic rainfall and low agro-chemical inputs during Cultivation. This is even more evident in developing countries where agro-chemicals and irrigation cultivation costs are beyond the reach of peasant farmers (Erpelding, 2008; Rao, *et. al.*, 2010).

1.6.2 Sorghum grains

Sorghum grains remain an important cereal in sub-Saharan Africa particularly in regions threatened by desert encroachment scourge. Sorghum may be cultivated in marginal land where other cereals do not grow very well. The grain production output in sub-Saharan Africa is estimated at 26 million metric tons annually with Nigeria being the leading producer in the whole of Africa and second worldwide (Adebiyi *et al*., 2005). However, commercial scale utilisation of sorghum grain in Nigeria is very limited; hence, the value chain is grossly under developed. But following

government's increase on import tariff of barley, sorghum grains have been gaining commercial significance as it is used as adjunct in malting and brewing industry. In 2011, it is estimated that over 200,000 metric tons of sorghum grain is consumed in the malting and brewing sector and this is an improvement over the 120,000 metric tonnes reported for year 2009 (AATF, 2011; FMARD, 2012). Sorghum grains are normally an oblong caryopsis consisting of pericarp (comprising epicarp, mesocarp and endocarp), germ and endosperm portions respectively (Fig. 1.9). Beneath the pericarp is the testa layer, which may contain tannin that gives the grain a slight acidic taste. High tannin concentration levels may render the grain poor in terms of nutritional value; tannins bind with proteins and inactivates enzymes during digestion or mashing. The palatability of high tannin grains is very unpleasant to both humans and animals. This is why the grains are sometimes known as "bird proof" sorghum grains because even birds do not feed on such grains (Dillon, *et. al*., 2007; Prasad and Dhanya, 2011). The germ is normally devoid of starch but rich in B-complex vitamins, lipids, soluble sugars as well as albumin and globulin proteins respectively. The lipid content of sorghum grain is normally higher than that of rice, millet, wheat and cassava but lower than found in maize and oat for example (NIIR, 2006; de Mesa-Stonestreet, 2010). The endosperm constitutes the larger fraction of the kernel with starch contents typically in the range of 60 - 75% and proteins between 8 - 16%. de Mesa-Stonestreet (2010) suggested that the starch granules normally form a matrix structure with the prolamin proteins in the endosperm (de Mesa-Stonestreet, 2010). The α-kafirin (prolamin) proteins are arranged within the starch granule's inner core and are surrounded by the β-kafirins and γ-kafirins fractions, respectively (Fig. 1.9).

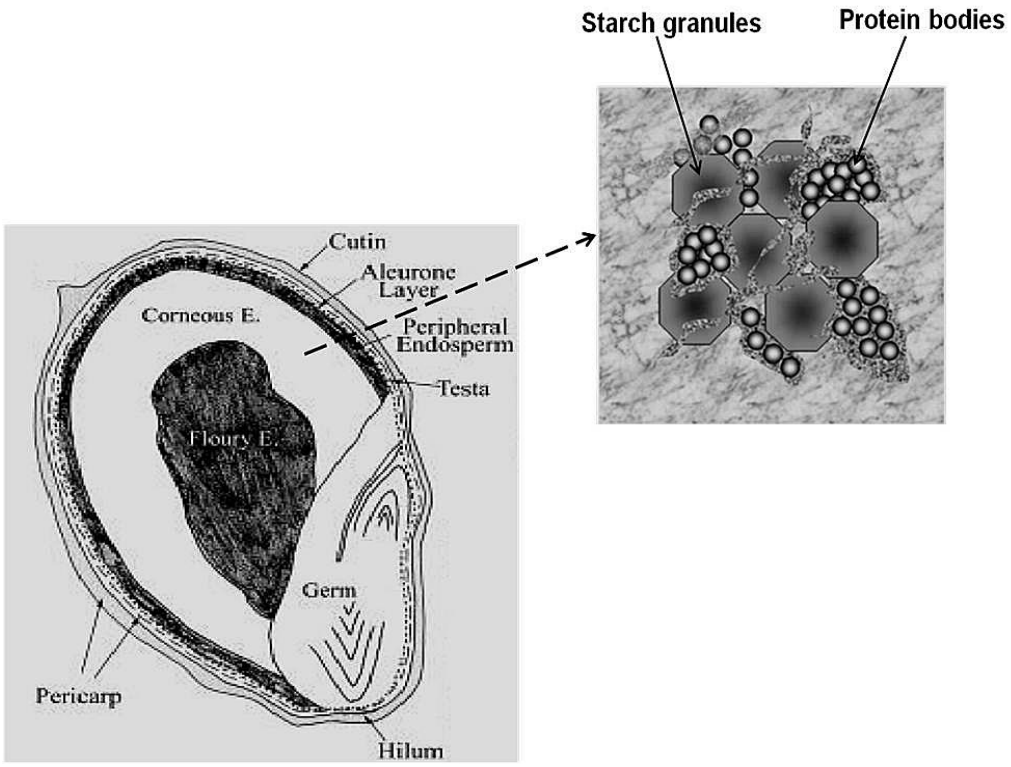

Fig. 1.9 Sorghum grain structure. A cross section of sorghum grain and a section of the endosperm showing typical starch-protein matrix structure.

During liquefaction and saccharification of sorghum grain starch, the outer protein layers (i.e. the β-kafirins and γ-kafirins fractions) tend to form a net-type structure that hinders easy access of proteolytic enzymes to the α-kafirin fraction which is easily degradable relative to the γ-kafirins fractions (INSORTMIL, 2010; de Mesa-Stonestreet, *et. al.,* 2010). This phenomenon accounts for the lower digestibility efficiency of sorghum starch by up to 15-20% relative to maize starch. Therefore, sorghum grains are reported to usually contain some relative amount of resistant starch which digests very slowly when consumed as food. Hence, it is considered an excellent source of energy, because this slow digestion process delays hunger and provides satiety (Dicko *et. al.,* 2006; Wong, *et. al.,* 2009). Sorghum is generally low in gluten content, certain cultivars are even gluten-free, thereby significantly reducing risk levels of gluten enteropathy i.e. Celiac disease in humans (Liu *et. al.,* 2012). Finally, red or brown coloured sorghum grains (Fig. 1.10) are typically rich in certain

phenolic compounds that are good antioxidants which could help reduce the risk of certain cancers (Dykes and Rooney, 2006).

Fig. 1.10 Some examples of sorghum grain cultivars. Typical varieties of sorghum crop heads and grain colours. Courtesy: CIAD (Center for Integrated Agricultural Development), Nigeria.

1.6.3 Sorghum stalk juice

Sweet sorghum stalk juice contains variable amount of sugars, proteins and starch depending on cultivar type, crop harvesting time, and cultivation location (Ritter *et. al.* 2007; Massoud and El-Razek, 2011). Typical sugars found in sorghum stalk juice are predominantly glucose, sucrose and fructose, while maltose, maltotriose and maltodextrins may be present in lesser concentrations (Almodares, *et. al*., 2008; Bridgers, *et. al*., 2011). Sorghum juice sugar levels increase as a function of crop cultivation duration and will normally peak as the grains approached maturity stage (Wortmann and Regassa, 2011). Reddy *et al.* (2010) reported sweet sorghum juice can contain 13% to 24% sugar Brix. Brix is a measure of total soluble sugars and the

starch level present in the juice on the basis of light refraction. Sorghum stalk juice may be broadly classified as either "syrup type" or "sugary type" depending on their sucrose, glucose and fructose sugars concentration levels. For example, juice with sucrose as its predominant sugar is classified as "sugary type" while juice having glucose and fructose as the predominant sugars is known as "syrup type" (Bitzer and Fox, 2000; Almodares, *et. al.,* 2008; Mazumdar, *et. al.*, 2012). Traditionally, when harvesting sorghum for syrup production, the stalks are usually topped to remove the panicle and stripped off the leaves before crushing the stalks for juice extraction. This is because certain chemicals from the leaves and panicle may impair the juice taste quality and degrade the final syrup quality. Sorghum stalk juice syrup is high in calorific value relative to molasses and maple syrup and is rich in mineral nutrients such as zinc, phosphorus, and potassium as well as vitamins such as riboflavin and B-6 that are necessary for human well being (Kuepper, 2009; Mazumdar, *et. al.,* 2012).

In spite of some sorghum cultivars stalk juices being rich in sucrose sugars similar to sugarcane, the former is less attractive for refined sugar production than the latter, this is because sorghum juice contains relatively high fibre, starch and invert sugars (i.e. glucose and fructose) which are not suitable ingredients for crystal sugar refining (Almodares and Hadi, 2009), Nevertheless, the sorghum juice is a suitable fermentation feedstock like cane juice because invert sugars are fermentable by yeasts and contains higher yeasts nitrogenous compounds than cane juice (Andrzejewski *et al.,* 2013). Extraction of sorghum stalk juice destined for ethanol fermentation could be done without stripping the stalk's leaves (unlike when the juice is destined for syrup production). The leaves and panicle may wilt before the juice extraction. The juice is then decanted after at least two hours of settling in holding

tank to remove sediments. Finally, alpha-amylase enzymes may be added during preheating of the juice to hydrolyse soluble carbohydrates. Removal of stalk panicles and leaves is of less importance for fuel ethanol production since taste is not an issue as is the case when the juice is destined for syrup production for human consumption (Wortmann and Regassa, 2011). However, for improved fermentation performance of the sorghum juice substrates, further processing such as filtration, sterilization, autoclaving, re-concentration, pH adjustment among others may be considered (Kim *et al*., 2012).

1.6.4 Sorghum bagasse

Sorghum plant stalks are largely composed of bark and pith. The pith is the central portion of the stalk and is made up of soft white finely divided holocellulosic material suitable for juicy sugar accumulation, the bark, which houses the pith is made up of thick and hard membranous tissue cover by epidermis surface on the outside, waxy white powdery substances normally covered the epidermis surface (Billa, *et. al.*, 1997; Hills *et al*., 1999). The waxy powder layer prevents or minimises moisture loss due to evaporation from plant stalks during dry climatic conditions (such as during drought) and prevents water from entering the pith when crops encounter water logged conditions (Kimber *et al.* 2013). While the pith is usually richer in soluble sugars, the bark is normally richer in cellulose, hemicellulose and lignin polymers (Table 1.10). Therefore, the bark constitutes the tough fibrous material that renders protection to the pith (Billa *et al*., 1997).

Table 1.10 Typical sorghum stalk chemical composition (% dry wt. basis)

Fraction	Pith	Bark
Cellulose	8.7	19.2
Hemicellulose	6.3	17.5
Lignin	0.6	8.8
Sucrose	67.4	32.2
Glucose	3.7	2.4
Ash	0.2	0.5

Source: Billa *et. al.* (1997)

Sorghum leaves consists of sheath and blade. The long sheath is normally adhered to the internode and the smooth surfaced broad blade is covered with a white waxy powder similar to that of the stalk bark. The wax is meant to serve a similar function of moisture loss prevention from leaves due to evaporation (Ritter, *et. al.*, 2007; Ghahraei, *et. al.*, 2008). The average weight of fresh leaves from a single plant cultivar varies between 150–250 g. Sorghum leaves are rich in proteins with sugars ranging from 3-5%, thus providing sorghum bagasse its nutritious value as livestock feed (Evert, 2006; Zhao, *et. al.*, 2009). Sorghum stalks are typically utilised in fence thatching in villages as well as for feeding livestock, the dried stalks are also used as domestic cooking fuel source (Ritter *et al*., 2007). By and large, summary use of whole sorghum biomass is presented in Table 1.11 and Fig. 1.11 shows sorghum stalks dried in preparation for local fence thatching.

Table 1.11 Possible potential uses of sweet sorghum crop

Crop part	***Possible use options***
Grains	Livestock feed, food, and 1st generation bioethanol.
Juice	Syrups, sugar, and 1st generation bioethanol.
Bagasse	Livestock feed, pulp, bio-energy generation, compost, fertilizer and 2nd generation bioethanol.
Leaves, panicles etc	Livestock feed, pulp, bio-energy generation, compost, fertilizer and 2nd generation bioethanol.

Source: Koppen *et al.* (2009)

Fig. 1.11 Sorghum stalks arranged for sun-drying. Sorghum stalks from previous harvest season being sun-dried for fencing work (Kaduna, 2012).

1.7 Background of this reserach

Nigeria is a country of over 160 million people with landmass of about 923,770 square kilometres, of which about 72% of the total area is cultivable, but currently less than 30% is under cultivation (Abila, 2010). Alhough Nigeria is regarded as the 9th largest oil exporting country worldwide, it depends on imports to satisfy its domestic demand for refined petroleum products such as gasoline, kerosene and diesel (Ogundari *et al.,* 2012). Consequently, oil and gas activities in the country are more or less limited to exploration and exploitation activities with very minimal refining activities. Thus, very limited job opportunities are available to Nigerians in this oil and gas sector. In fact, less than 1% of the country's population are employed

in this sector despite its huge employment potential in terms of refining operations. Furthermore, the oil reserves are all located within the southern region of the country; this region constitutes less than 15% of the country's total population; thereby geographically limiting employment opportunities and direct economic benefits relating to oil and gas activities for the vast majority of Nigerians from other regions that comprise over 80% of the country's population (Azih, 2007). Agriculture used to be the mainstay of the Nigerian economy particularly in the northern region which has over 70% of the total arable land. It provides direct employment to over 70% of the national population prior to the "oil boom era". However, over the past decades the agricultural sector has suffered immeasurable neglect from successive governments because of "easy to get" oil money. This has led to the "collapsing" of the agricultural sector thereby creating millions of unemployed Nigerians with resultant massive rural to urban areas migration in search of jobs (Ohimain, 2010; Agboola *et al*., 2011). Recently, as part of efforts by government to revamp the agricultural sector to its "glorious days", the Nigerian oil and gas sector is intended to be integrated with the agricultural sector whereby, while the former produces gasoline and diesel, the latter produces bioethanol and biodiesel for E10 and B20 liquid transport production fuels.

In addition to Nigeria having an observer status to the Rio's 1992 Kyoto protocol on climate change, the rapidly increasing demand for liquid transport fuel in the country has motivated the government to incorporate bioethanol into its transport fuel energy supply (NNPC, 2007). Bioethanol was introduced into the Nigerian transport sector as E-10 fuel through Nigerian Biofuels Policy and Incentives which was articulated in 2007 by the Nigerian National Petroleum Corporation (NNPC). Annual bioethanol demand for E-10 fuel blending is estimated at 1.3 billion litres annually and it is

projected to reach 2 billion litres by 2020, currently, over 95% of the national bioethanol demand is met through importation. Thus, the main thrust of the policy is to integrate the agricultural sector with the oil and gas sector whereby the former produces 10% of bioethanol required for blending with 90% gasoline to produce E-10 fuel blend (Galadima *et al.,* 2011). In part, the policy is aimed to firmly establish an ethanol industry that will solely rely on local agricultural products as feedstock sources, thereby creating added value in the agricultural chain. According to the NNPC (2007), the specific benefits to be derived from the policy include;

1. The diversification of the nation internally generated revenue sources from additional taxes to be generated from the emerging bioethanol sector.

2. Creation of sustainable job opportunities for Nigerians and wealth creation for rural farmers, and by extension the communities.

3. Improving agricultural benefits by advancing farming techniques and mechanisation.

4. Ensuring energy supply sustainability and reduced dependence on fossil fuels as well as reduced GHG emissions from automobiles exhaust pipes.

Cassava, cane molasses, sugarcane and sweet sorghum stalk juice are the bioethanol production feedstocks mentioned in the Nigerian bioethanol policy programme (NNPC, 2007). Experts have argued that these are food crops and using food crops as bioethanol feedstock will be in direct conflict with the government's objectives of providing national food security (Ogundari *et al*., 2012: Ohimain, 2013). However, the sorghum plant is a high biomass yielding crop with strong resistance to several harsh environmental conditions and can be grown with relatively low water

requirements and agrochemical applications. All of these positive agronomic attributes of sorghum are in addition to having potentially 2-3 crop cycles per annum (Almodares *et al.*, 2009). Sorghum is cultivated on nearly 7 million hectares of farmland in Nigeria with annual grain production turnover of about 9 million tons and dry crop residue wastes of 2-3 million tons annually (Ogbonna, *et al.,* 2003; Ohimain, 2013). The sweet sorghum varieties may be cultivated to produce grains, stalk juice and bagasse respectively, therefore, sorghum is envisaged to potentially be a sustainable feedstock source for the emerging Nigerian bioethanol sector, while the grains may be utilised for brewing operations, the stalk juice may serve the syrup making industry and the green wastes may be partly utilised for bioconversion to ethanol. In addition, degraded or spoilt sorghum grains that are constantly generated in Nigeria due to poor storage facilities or residual grains from birds or pests invaded farmlands may also be utilised in the bioethanol sector.

1.7.1 Aim of this research

In this Ph.D work, the potential of sorghum crop as feedstock source for bioethanol production via juice, crude grains and bagasse platforms was investiged. Refer to Fig. 1.12 for a schematic representation of this programme of research.

1.7.2 Objectives of the study

The objectives of this study included:

- ✓ To compare the fermentation performance of three Nigerian sorghum cultivars grown under rain fed conditions without chemical fertilizer application.
- ✓ To assess the impact of cultivation location on sorghum physico-chemical composition and subsequent fermentation performance.
- ✓ To investigate the potential for bioconversion of degraded sorghum grains and stalk juices to ethanol via low cost fermentation conditions.

- ✓ To investigate the potential for utilising whole sorghum crop residue in bioethanol production via low cost pre-treatment methods.

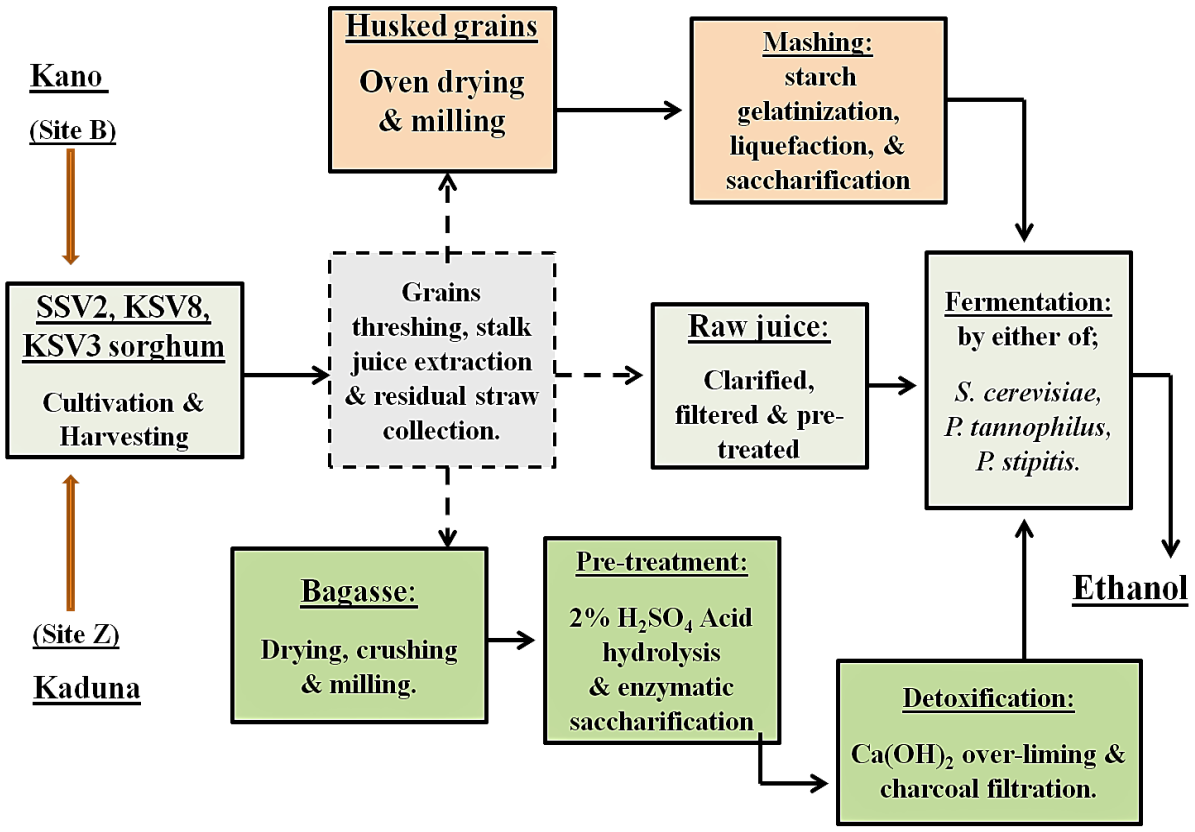

Fig. 1.12 Schematic representation of the experimental approach described in this Ph. D thesis. A process flow chart showing bioconversion routes of sorghum stalk juice, grains and bagasse as adopted in this study.

CHAPTER TWO

Impact of cultivation location on sorghum stalk juice fermentation performance

2.0 Introduction

Sorghum is a water use efficient cereal that thrives well under varied climatic and environmental conditions. It is a C-4 plant with efficient photosynthesis ability. Therefore, warmer and drier climates favour higher biomass yields (Almodares *et al.,* 2008). Sweet sorghum stalk juice contains variable amounts of sugars, proteins and starch depending on the cultivar type, harvesting time and cultivation location (Massoud and El-Razek, 2011). Typical sugars found in sorghum stalk juices are predominantly glucose, sucrose and fructose, while maltose, dextrins, maltotriose and other oligosachharides may be present in minute concentrations (Almodares and Hadi, 2009).

Sorghum stalk juice may be broadly classified as either "syrup type" or "sugary type" depending on the concentration levels of sucrose, glucose and fructose present in the raw juice. For example, juice with predominantly sucrose is classified as "sugary type" while juice having glucose and fructose as the predominant sugars are known as "syrup type" (Bitzer and Fox, 2000; Mazumdar *et. al.*, 2012). The "syrup type" juices are typically utilised in syrup production commercially, while the "sugary type" is traditionally consumed as snack food by chewing the fresh sorghum stalk. However, both juice types are potentially suitable substrates for bioconversion to ethanol (Bitzer and Fox 2000; Nasidi *et al.*, 2010). Investigations into low-cost nitrogen sources to enrich sorghum juice nutrient content for efficient bioconversion

to ethanol have been extensively reported in the scientific literature. For example, studies have reported the use of commercially available nutrients such urea, peptone, yeast extracts, dried spent yeasts, and di-ammonium phosphate (DAP) as important nitrogen sources as supplements for fermentation substrates. Nitrogeneous compounds are essential in fermentation substrates to maintain healthy and viable cells growth during fermentation. Therefore, enrichment of the fermentation substrates with yeast extract, diammonium phosphate and/or dried spent yeasts (DSY) significantly reduces yeast lag time and improve yeast fermentative capacity. This leads to increase ethanol yield by up to 20% or more and with additional benefit of shorten fermentation time (Serna-Saldivar, 2011; Laopaiboon and Laopaiboon, 2012; Yu *et. al.,* 2012; Zhao *et. al.,* 2012). However, supplementation of sorghum juice as fermentation substrate for commercial scale ethanol production contributes significantly to the overall production process running costs (Davila-Gomez *et al*. 2011; Imam and Capareda, 2012; Zhao *et. al.,* 2012).

Therefore, the objective of the research work presented in this Chapter was to investigate the impact of cultivation location on sorghum juice composition and the potential for utilising high nitrogenous sorghum juice (with relatively low levels of fermentable sugar), as low-cost nitrogen sources for high-sugar sorghum juice fermentations. The experimental approach comprised obtaining crude juice samples of SSV2, KSV8 and KSV3 sorghum cultivars each grown at both Kano (site B) and Kaduna (site Z) in Nigeria. The juices were then blended at appropriate volume ratios to obtain relatively nutrient-rich juice blends for efficient bioconversion to ethanol.

2.1 Materials and Methods

2.1.1 Crop cultivation and juice extraction

Nigerian sorghum cultivar seedlings SSV2, KSV8 and KSV3 were cleaned, treated with metalaxyl fungicide chemical (Apron Star™, Nigeria) and planted in Kano (site B) and Kaduna (site Z), Nigeria (Fig. 2.1). Table 2.1 presented some reports on physical and morphological soil properties of sites B and Z (Giginyu and Fagbayide, 2009; Oduze and Kureh, 2009). However, KSV3 seedlings in Kaduna were lost to bird invasions. The crops were cultivated under rain-fed conditions with only cow dung manure application. The SSV2 sorghum cultivar was harvested at 11 weeks after planting date, when the grains were observed to have reached soft-dough stage. However, KSV8 and KSV3 sorghum cultivars were harvested 16 weeks after planting date, when their grains also reached soft-dough stage. This is because maximum extractible sorghum stalk juice is obtainable when the grains were at the soft-dough stage (Almodares *et al.*, 2008). One hundred freshly stripped and cleaned stalks of SSV2, KSV8 and KSV3 which are randomly selected from each corner of field are prepared for juice extraction. Without further slicing, cutting or peeling of stalks, 5-7 pieces of stalks are manually fed into the horizontal 2-roller mill at a time (Ohaus®, Swizerland). The roller diameter is 10-inches each and the machine is 7.5 kW electric powered with about 85% juice extraction efficiency. The fly wheel rated speed is 3600 rpm. Fig. 2.2 showed the stalk juice extraction process. The freshly extracted juices (6 L) each were separately added into gravity settling tanks and allowed to clarified by gravity settling for about 3 h. By this process, heavy suspended particles settled to the bottom and the lighter floating matter are later decanted. Finally, the clarified juices were filtered by running through an 8 litre volume filter bucket fitted with a polyester filter bags™ at the top (SS Bolting Cloth,

China). Finally, the filtered juices were pasteurized by heating at 65^{o}C with constant stirring for 2 h. The pasteurization was aimed to reduce microbial contamination in the juice. It also allowed dissolved impurities to precipitate to the bottom. However, pasteurisation of sorghum juice above 70^{o}C may cause juice starch to start gelling (Mazumdar *et al.,* 2012). The juice was finally sterilised by adding into a 4 litre aluminum pot and placed in a steam steriliser™ at 124^{o}C (Dacheng, China) for 15 min. Juice samples were stored at -20^{o}C until further analysis.

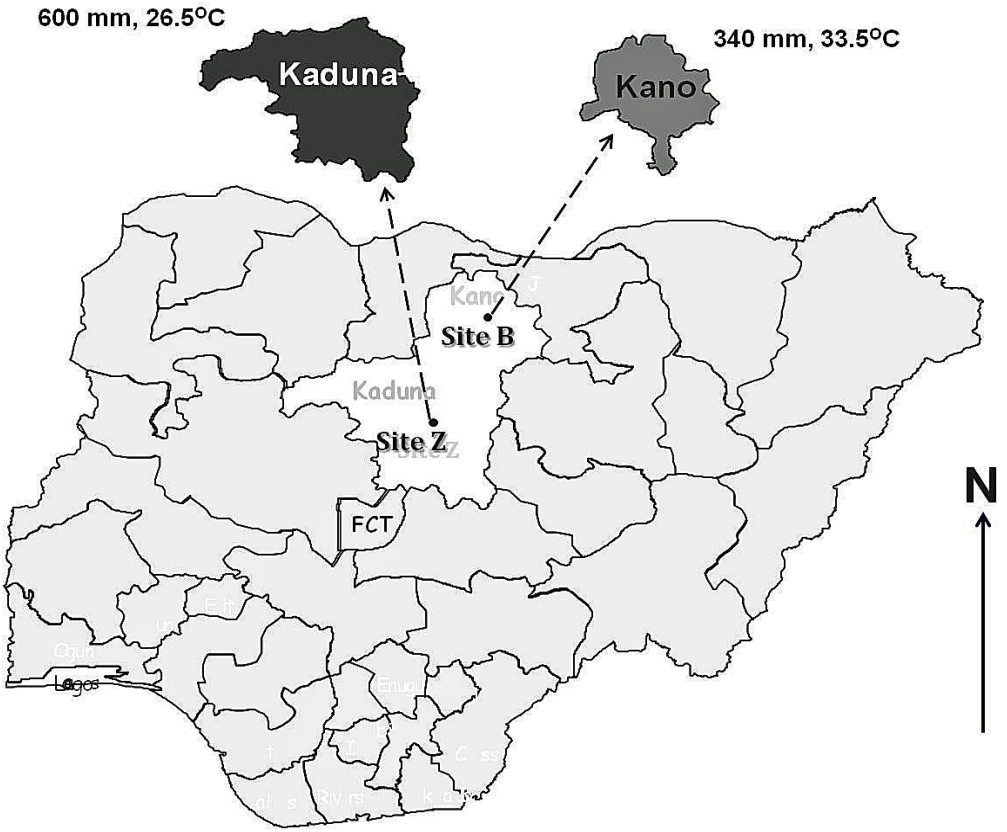

Fig. 2.1 Map of Nigeria showing chosen cultivation locations. Map of Nigeria showing site locations B (coordinates: 11.33^{O}N, 8.23^{O}E) and Z (coordinates: 11.10^{O}N, 7.38^{O}E) as well as the climatic conditions where SSV2 and KSV8 sorghum cultivars were grown for the purpose of this study.

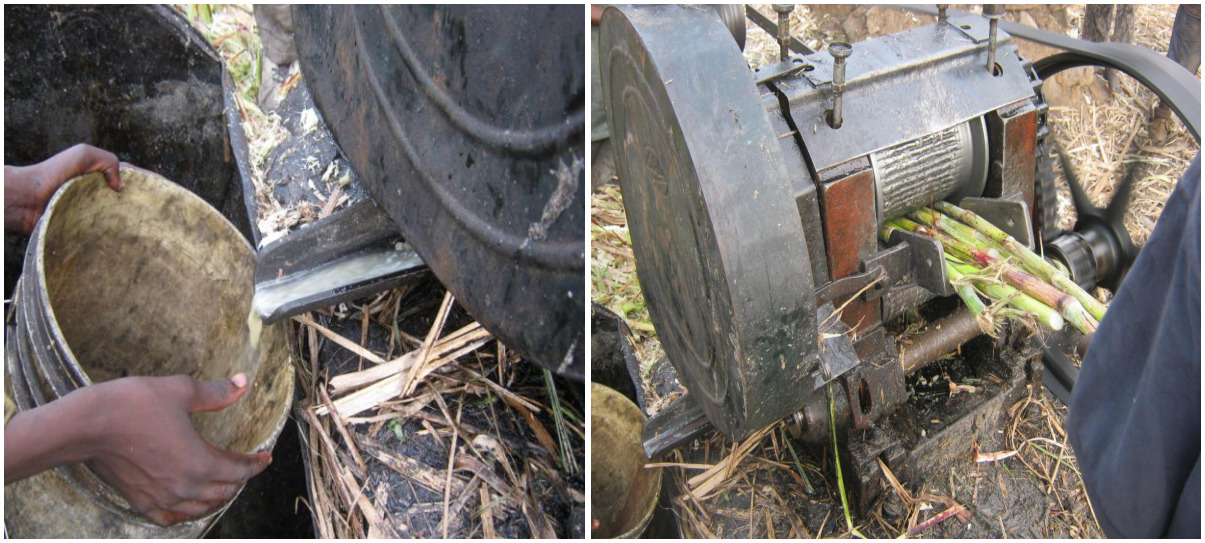

Fig. 2.2 Sorghum stalk juice extraction by milling. Sorghum stalks juice extraction process. SSV2, KSV8 and KSV3 sorghum stalk juices were extracted with a roller mill (Ohaus, Switzerland). Randomly selected 100 fresh stalks of each cultivar grown in Kano and Kaduna were milled. The extraction process for each sorghum cultivar batch (100 stalks) was repeated in triplicate.

Table 2.1 Soil physical and morphological properties of Kano and Kaduna sites

Parameters	**Site B**	**Site Z**
pH	5.0	5.2
Org. C (g kg^{-1})	0.38	3.3
Total N (g kg^{-1})	0.08	0.53
Avail. P (mg kg^{-1})	0.56	1.8
Exchangeable bases (C mol kg^{-1})		
Ca	0.27	1.80
Mg	0.08	0.36
Na	0.30	0.05
K	0.19	0.33
Exch. Acidity (Al^{3+} H^{+})	0.24	0.10
CEC	1.08	4.0
Soil physical properties (g kg^{-1})		
Sand	78	46
Silt	12	40
Clay	10	14

Source: Giginyu and Fagbiyide (2009); Oduze and Kureh (2009).

2.1.2 Juice compositional analysis.

a. Total Starch: Megazyme's K-TSTA standard protocol for total starch determination from juice was employed with the following modifications:

i. Fresh crude juice (5 mL) was filtered through Whatman GF/A Glass fibre filter papers.

ii. Aliquots (2 mL) of the filtered juice were dispensed into 38 mm X 200 mm borosilicate glass test tubes to which 8 mL of 95 % v/v ethanol was added. Final solutions were vortexed for 2 min then allowed to stand at room temperature for 30 min, prior to centrifugation at 3800 rpm for 10 min.

iii. Supernatants were dispensed into sterilized glass tubes and the volume topped to 3.9 mL with acetate buffer (100 mM acetate, pH 5.0) to which 0.1 mL of diluted *amyloglucosidase* (AMG) solution (Megazyme, Northern Ireland) was added. Final solutions were vortexed for 1 min and incubated in a water bath at 50°C for 30 min before 0.1 mL aliquots were transferred to 16 mm x 100 mm glass test tubes.

iv. GOPOD Reagent solution (3 mL) (Megazyme, Northern Ireland) was added to the 0.1 mL of solution from (iii) above, and the final solution was incubated in a water bath at 50°C for 20 min. The starch content was determined by reading the absorbance of the final solution at 510 nm using a Genesys™ spectrophotometer (Thermo Spectronic®, USA).

Note:

i. The D-Glucose control sample comprised: 0.1 mL of D-glucose standard solution and 3.0 mL of GOPOD Reagent only.

ii. Reagent Blank solutions (control) comprised: 0.1 mL distilled water and 3.0 mL of GOPOD Reagent.

b. Crude Protein: Sigma-Aldrich's Bradford standard protocol for protein determination was employed with the following modifications:

i. Coomassie reagent (3 mL) was added to 0.1 mL of pre-diluted juice aliquots (1:10 dilution).

ii. The final juice solutions were vortexed for 2 min and incubated at room temperature for 45 min.

iii. The absorbances of the incubated solutions (from ii above) were read at 595 nm using a spectrophotometer.

iv. Protein concentration levels were determined against a pre-prepared standard calibration curve.

c. **Free Amino Nitrogen (FAN):** Total FAN was determined by K-Large 02/11™ (yeast available nitrogen, YAN) and K-PANOPA 02/11™ (primary amino acid nitrogen, PAN) assay kits (Megazyme, Northern Ireland) following the manufacturer's standard protocol. While the K-LARGE kit is essentially use to determine L-arginine/Urea/Ammonia, the K-PANOPA kit is use to determine total Primary Amino Nitrogen (PAN) comprising range of amino acids in samples.

d. **Fermentable Sugars (HPLC):** Aliquots of sorghum juice (1 mL at 1:10 dilution) were filtered through 0.22 µm micro syringe filters into vials and made up to 2 mL with 5 mg/mL *meso*-erythritol solution. The final solutions were vortexed and placed in an HPLC autosampler (Spectra-physics, USA) and sugars separated with a 300 mm × 7.8 mm REZEX RPM-monosaccharide Pb+2 (8%) column™ (Phenomenex, USA). The operating parameters for the HPLC are provided in Table 2.2. Sugars were detected using refractive index

and quantified using HPLC software (CSW32 version v.1.4 chromatogram software from DataApex®, USA).

Table 2.2: HPLC operating conditions

Parameters	Conditions
Mobile phase	De-gassed dH_2O
Flow	0.6 mL/min
Temperature	80°C
Pressure	400 psi
Detector	RI (refractive index)
Injection volume	20 µL
Run time	20 min
Internal standard sugar	Meso-Erythritol

Amino Acids Composition: Total free amino acids composition was determined courtesy of Heriot-Watt University Edinburgh. Fresh juice 2 (mL) samples were filtered through 0.22 µm filters into HPLC-grade vials and placed into HPLC equipment. The analysis was performed by gradient elution, high performance liquid chromatography (HPLC), using fluorescence as a means detection (Chiba *et al*., 2012). Detailed description of the HPLC instrument and column used for the amino acid profile test are:

Instrumentation:

Gilson 231 autoampler with **40l** dilutor, Rheodyne 7010 injector with 20 µl loop, Gilson 302 and 306 pumps with 5SC pump head, Gilson 802 Manometric controller, Gilson 811 C dynamic mixer, Gilson 715 data handling package, Phenomenex Degassex (degassing unit) Model DG4400, Jasco FP 1520 fluorescent detector.

Column:

Phenospere Next, 5µ, C18, 150 × 4.6 mm (Phenomenex, U.K)

2.1.3 Raw juice blending and enzymatic hydrolysis.

Two aliquot volumes of fresh stalk juice (70 mL) from SSV2Z sorghum were each topped to 100 mL by separately adding fresh juice from SSV2B and KSV3B sorghums (30 mL) each, respectively. Furthermore, SSV2B fresh juice (70 mL) was also topped to 100 mL by adding fresh juice of KSV3B (30 mL). Subsequently, three 100 mL juice blends (SSV2Z + SSV2B), (SSV2Z + KSV3B) and (SSV2B + KSV3B) were obtained respectively. These juice blends (100 mL) each were hydrolysed with the addition of 30 μL of Promalt^{TM} 295 enzymes (β-glucanase/amylase/protease) courtesy Kerry Biosciences, Ireland. The enzymatic hydrolysis process of the juice blends involved initial incubation of juice blends at 50^{o}C for 60 min with 150 rpm orbital shaking, the resultant hydrolysates were further incubated for 30 min at 60^{o}C and finally allowed to cool to room temperature for subsequent fermentation by yeasts.

2.1.4 Raw juice fermentation.

Frozen raw juices were thawed to room temperature, filtered through glass fibre filters (Millipore®, Sigma Aldrich). Without pH adjustment, dilution or further sterilisation, *S. cerevisiae* cells (1.0 × 10^{7} cell/mL) were inoculated into 250 mL Erlenmeyer conical flask containing 100 mL fresh juice and fermented at 32^{o}C with orbital shaking at 130 rpm. Samples were withdrawn every 24 h for alcohol determination by gas chromatography using a Shimadzu GC-MS model QP2010 (Shimadzu, USA). Absolute propan-1-ol (50 µL) was added into 4.95 mL fermentation broth and vortexed. The solution was placed on the GC-MS tray for analysis. The brief analysis instrumentation and conditions are:

The column is an Rtx®-BAC2 (30 m × 0.32 mm by 1.2 µm (Restek, USA). Sample injection time is 0.05 min, oven temperature is 60^{O}C. The carrier gas is Nitrogen. The

GC-cycle time is 15 min and data sample time is 6 min. The mass range is 29 to 300 m/z.

2.1.5 Yeast seed culture preparation.

Two loopfuls of industrial *Saccharomyces cerevisiae* (DCLM) yeast strain (courtesy of Kerry Biosciences, Menstrie, Scotland) were inoculated into 400 mL YEPD media comprising 4.0% (w/v) bacteriological peptone, 2.0% (w/v) yeast extract and 4.0% (w/v) glucose respectively. Cultures were incubated at 32^{o}C with orbital shaking at 150 rpm for 20 h.

2.1.6 Statistical analysis

Significant difference between means was tested by ANOVA using Turkey method by Minitab™ 16 statistical software (MINITAB©, USA). Means that do not share a superscript letter (a-e) within same rows are significantly different ($p \leq 0.05$) based on grouping information using Tukey method at 95% simultaneous confidence interval.

2.2 Results and Discussion

2.2.1. Juice compositional analysis

The results presented in Table 2.3 indicated that despite SSV2 having shorter cultivation duration than KSV8 and KSV3, the former has higher juice yield than either of the latter. Furthermore, Kaduna location favoured higher juice yield relative to Kano. Data summarised in Table 2.3 further indicated that cultivation location significantly impacted ($p \leq 0.05$) on compositions of SSV2 and KSV8 juices. For example, while Kaduna appeared to favour high stalk juice sugar accumulation, Kano favoured higher stach juice accumulation. The observed significant difference $p \leq 0.05$ in the juice starch compositions may be partly related to the agronomic attributes of sorghum crop being a C-4 plant which has a high photosynthetic

efficiency. Therefore, Kano with warmer and drier climate favoured higher juice starch formation (i.e. higher rate of glucose polymerisation to starch) while Kaduna tended to favour higher sugar accumulation in juice (Almodares and Hadi, 2009). With regards to juice nitrogenous contents, Table 2.3 results inidcated that while KSV8 juice proteins are not significantly different p ≤0.05 between Kano and Kaduna, the SSV2 juice proteins are significantly different p ≤0.05. However, the FAN content of the juices is significantly different (p ≤0.05). The variations in the juice nitrogenous compounds may be related to results in Table 2.1, which indicated variations in the Nitrogen, Phosphorus and potassium concentrations in soil compositions of Kano and Kaduna. Furthermore, it appeared that the total amino acids concentrations presented in Table 2.4 are higher than corresponding total FAN concentrations in Table 2.3. These were attributed to the specific limitations of K-LARGE/K-PANOPA Megazymes kits (used to determined total FAN) in detecting certain specific amino acids (Megazymes, 2011).

Principally, FAN comprises of ammonium (NH_4^+) ions, small peptide polymers and amino acids (Feldmann, 2012). Amino acids are essential nutrients for efficient yeast metabolic activities during fermentation. The uptake of amino acids by yeasts during fermentation will depend on the cells condition i.e. whether at lag phase, exponential or late growth phase (Walker, 1998; Bisson and Butzke, 2000; Nie *et. al.*, 2010). Table 2.4 show SSV2, KSV8 and KSV3 juices have all the essential amino acids necessary for effective yeast metabolism and this is in addition to the juices having more than the minimum 150 mg/L FAN level required for efficient fermentation process (except for KSV8Z) as presented in Table 2.3. While the Group 1 amino acids are not synthesized by yeasts but are required for uptake at the onset of fermentation. The Group 2 and "Other group" amino acids can be synthesized by

yeast cells during fermentation and are normally assimilated sequentially as the fermentation progresses (Lekkas *et al.*, 2007; Lucie *et. al.*, 2008).

Table 2.3 Compositions of SSV2, KSV8 and KSV3 sorghums raw stalk juices

Item	**Kano**			**Kaduna**	
	SSV2	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Juice yield (L/ ha)	25024^{b} ±20.4	23304^{d} ±4.93	22570^{e} ±16.26	25596^{a} ±13.32	24536^{c} ±9.07
Total starch (g/L)	0.97^{b} ±0.07	0.51^{e} ±0.03	1.69^{a} ±0.10	0.64^{c} ±0.01	0.37^{d} ±0.02
Total protein (g/L)	1.58^{c} ±0.10	1.08^{d} ±0.07	2.18^{a} ±0.05	1.82^{b} ±0.04	1.03^{d} ±0.06
Total FAN (mg/L)	224^{c} ±1.14	191^{d} ±1.43	365^{b} ±3.21	325^{a} ±3.22	134^{e} ±1.52
Sucrose (g/L)	102.71^{b} ±3.76	36.41^{d} ±2.11	60.46^{c} ±2.14	113.93^{a} ±1.88	55.67^{c} ±1.39
Glucose (g/L)	27.58^{b} ±2.03	19.73^{c} ±0.83	30.98^{a} ±1.53	32.07^{a} ±1.14	21.76^{c} ±1.18
Fructose (g/L)	13.69^{b} ±1.54	9.67^{d} ±0.13	12.49^{c} ±1.76	15.50^{a} ±0.34	10.52^{e} ±0.96
TOTAL SUGARS	**143.99^{a} ±3.27**	**65.81^{b} ±2.81**	**103.93^{c} ±3.18**	**161.50^{d} ±3.36**	**87.96^{e} ±3.53**

SSV2, KSV8 and KSV3 sorghums were cultivated in Kano and Kaduna (Nigeria) under rain fed conditions and without chemical fertilizer applications. While SSV2 was harvested 11 weeks after planting, KSV8 and KSV3 were harvested 16 weeks after planting. Means on the same row that do not share same superscript letter (a-e) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

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Table 2.4 Amino acids composition of SSV2, KSV8 and KSV3 sorghum juices

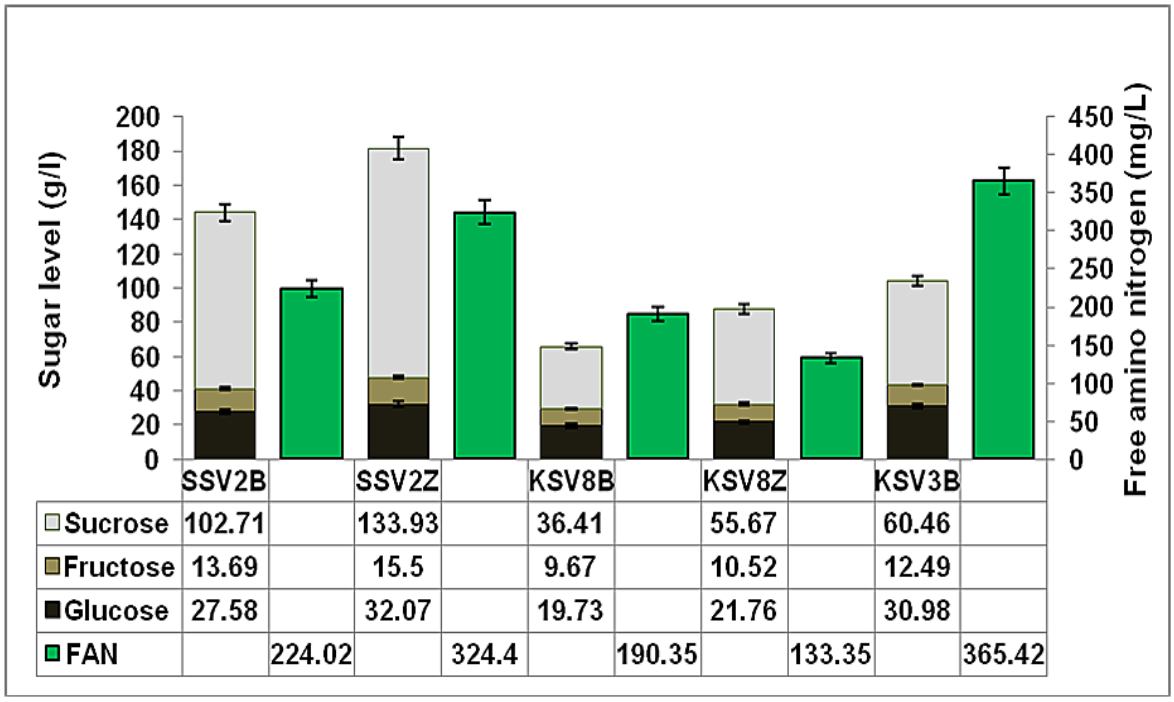
	Kano			Kaduna	
Amino acids	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Group 1 (μmole/mL)					
aspartic acid	1.141^{a} ±0.011	0.631^{c} ±0.007	0.750^{b} ±0.002	0.730^{b} ±0.009	0.530^{d} ±0.006
glutamic acid	0.444^{b} ±0.080	0.403^{c} ±0.005	0.470^{a} ±0.001	0.538^{a} ±0.008	0.462^{b} ±0.007
asparagine acid	6.410^{d} ±0.120	6.705^{c} ±0.070	11.355^{a} ±0.130	10.580^{b} ±0.020	3.885^{e} ±0.070
glutamine	3.145^{a} ±0.090	1.545^{d} ±0.070	1.840^{c} ±0.007	2.690^{b} ±0.080	1.595^{d} ±0.070
serine	0.956^{a} ±0.007	0.396^{b} ±0.007	0.405^{b} ±0.002	0.750^{d} ±0.005	0.345^{c} ±0.006
arginine	0.093^{a} ±0.004	0.055^{c} ±0.006	0.076^{b} ±0.001	0.082^{e} ±0.005	0.030^{d} ±0.006
threonine	0.391^{a} ±0.007	0.196^{c} ±0.007	0.194^{b} ±0.001	0.293^{e} ±0.004	0.125^{d} ±0.003
lysine	0.086^{b} ±0.005	0.048^{c} ±0.003	0.049^{d} ±0.000	0.076^{a} ±0.006	0.014^{c} ±0.003
Sub-Total	***12.664 ±0.234***	***9.977 ±0.025***	***15.214 ±0.011***	***15.738 ±0.103***	***6.985 ±0.157***
Group 2 (μmole/mL):					
histidine	0.071^{a} ±0.007	0.061^{b} ±0.003	0.040^{d} ±0.000	0.033^{c} ±0.002	0.023^{c} ±0.004
methionine	0.027^{a} ±0.004	0.013^{b} ±0.001	0.018^{c} ±0.000	0.020^{a} ±0.004	0.007^{d} ±0.003
isoleucine	0.264^{d} ±0.006	0.181^{e} ±0.006	0.293^{b} ±0.001	0.293^{b} ±0.004	0.133^{a} ±0.004
leucine	0.205^{c} ±0.008	0.147^{e} ±0.005	0.208^{c} ±0.001	0.275^{a} ±0.005	0.105^{b} ±0.007
phenylalanine	0.192^{e} ±0.008	0.086^{d} ±0.005	0.096^{c} ±0.000	0.085^{d} ±0.004	0.037^{a} ±0.007
valine	0.644^{e} ±0.003	0.354^{a} ±0.005	0.485^{b} ±0.001	0.674^{e} ±0.008	0.241^{c} ±0.007
Sub-Total	***1.402 ±0.008***	***0.841 ±0.005***	***1.138 ±0.008***	***1.380 ±0.003***	***0.544 ±0.001***
Other group (μmole/mL)					
glycine	0.109^{a} ±0.006	0.064^{b} ±0.005	0.104^{a} ±0.001	0.165^{d} ±0.006	0.076^{b} ±0.007
alanine	0.967^{d} ±0.004	0.434^{e} ±0.008	0.435^{e} ±0.003	1.063^{d} ±0.008	0.389^{e} ±0.005
proline	0.034^{c} ±0.008	0.031^{c} ±0.005	0.036^{c} ±0.000	0.134^{a} ±0.008	0.027^{c} ±0.005
tryptophan	0.226^{a} ±0.006	0.095^{b} ±0.004	0.126^{c} ±0.001	0.070^{d} ±0.004	0.042^{e} ±0.004
tyrosine	0.450^{b} ±0.005	0.231^{c} ±0.007	0.272^{a} ±0.004	0.181^{e} ±0.008	0.184^{e} ±0.010
Sub-Total	***1.786 ±0.007***	***0.853 ±0.019***	***0.972 ±0.005***	***1.613 ±0.002***	***0.717 ±0.009***
Grand Total	**15.852^{a} ±0.055**	**11.671^{b} ±0.114**	**17.324^{c} ±0.035**	**18.731^{d} ±0.046**	**8.246^{e} ±0.231**

The SSV2, KSV8 and KSV3 sorghums were cultivated in Kano and Kaduna (Nigeria) under rain fed conditions and without chemical fertilizer applications. While SSV2 was harvested 11 weeks after planting, KSV8 and KSV3 were harvested 16 weeks after planting. Means on the same row that do not share same superscript letter (a-e) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

2.2.2 Raw juice fermentation

The SSV2Z and KSV3B that contained higher amounts of glucose (Fig. 2.3) and Group 1 amino acids (Table 2.4) showed faster initial fermentation rates than SSV2B, KSV8B and KSV8Z (Fig. 2.4). With regards to ethanol yields, the SSV2Z juice having highest total sugar concentration shows highest corresponding ethanol yield followed by SSV2B. Equally, KSV8B with relatively lowest total sugars level yielded lowest ethanol concentration. Furthermore, KSV8Z and KSV8B substrates fermentations were completed within 24 h because most of the available sugars appeared to have been utilised along with corresponding ~83% and ~90% of FAN respectively (Table 2.5). With regards to KSV3B juice fermentation, all the sugars

were utilised along with ~75% of FAN after 24 h. However, SSV2Z and SSV2B juice fermentations were completed after 48 h, the respective FAN utilisation were ~66% and ~73% respectively. Residual sucrose was detected in the fermentation broth (Table 2.5), this suggested depletion of vital minerals and/or vitamins in juice substrates during the early stage of fermentation process thereby limiting yeast ability to synthesize *invertase* enzymes necessary for splitting of sucrose to glucose and fructose sugars that are assimilable to the *S. cerevisiae* yeasts (Lekkas *et al.*, 2008; Feldmann, 2012).



	SSV2B		SSV2Z		KSV8B		KSV8Z		KSV3B	
Sucrose	102.71		133.93		36.41		55.67		60.46	
Fructose	13.69		15.5		9.67		10.52		12.49	
Glucose	27.58		32.07		19.73		21.76		30.98	
FAN		224.02		324.4		190.35		133.35		365.42

Fig. 2.3 Sugars and FAN concentration levels of crude stalk juice. Fresh stalk juice sugars and FAN concentrations of SSV2, KSV8 and KSV3 sorghum cultivated in Kano and Kaduna (Nigeria). Sugars were analysed by HPLC while FAN was determined by K-PANOPA/K-LARGE Megazymes kits. Results are standard means of 3 replicates.

The SSV2B, KSV8B, KSV8Z and KSV3B raw juice substrates yielded ethanol concentrations that correspond to different values ranging from 23 to 68 g/L reported by Widianto *et al.* (2010) for various raw sorghum stalk juices fermented by *S. cerevisiae* and without exogenous nutrient supplementation. However, the SSV2Z juice substrate's ethanol yields of about 81 g/L is higher than the 73 g/L ethanol yield

reported by Gyalai-Korpos *et al.* (2008) for raw sorghum stalk juice also fermented without nutrient supplementation. Furthermore, the reported ethanol yield of SSV2Z (81 g/L) in this study compares favourably with 86 g/L ethanol yield reported by Zhao *et al*. (2012) from raw sorghum juice fermented by *S. cerevisiae* after supplementation with additional nutrients (Urea, DAP, and $MgSO_4$). Data in Table 2.5 indicated that juice substrates from Kaduna favoured higher ethanol yield over substrates from Kano, for example, SSV2 and KSV8 juice showed improved ethanol yield of about 20% and 30% in Kaduna. Furthermore, with regards to ethanol yield among SSV2, KSV8 and KSV3 juice substrates in Kano, SSV2 juice shows about 45% improved ethanol yield over KSV8 substrate. These results further highlighted the importance of cultivar type selection when contemplating sorghum for commercial scale ethanol production.

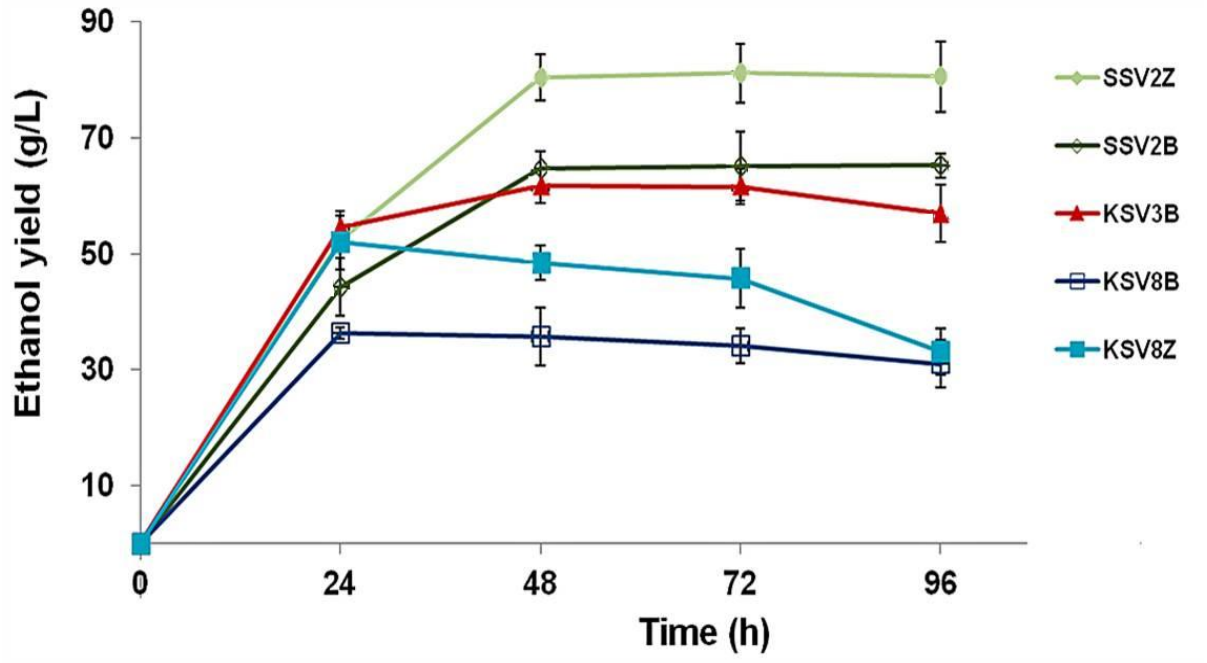


Fig. 2.4 Sorghum raw juice fermentation profile using *S. cerevisiae*. SSV2, KSV8 & KSV3 sorghum were grown in Kano and Kaduna (Nigeria). Raw juice (50 mL) was inoculated with 1.0×10^7 cells/mL of *S. cerevisiae* and incubated at 32^{o}C and 130 rpm. Samples were withdrawn after every 24 h for alcohol determination by GC-MS. Data are means of 2 independent replicates.

Table 2.5 Ethanol yields/residual sugars from fermented sorghum juice

Item	Kano			Kaduna	
	SSV2	KSV8	KSV3	SSV2	KSV8
Ethanol yield (g/L)	65.26[a] ±1.26	36.31[b] ±1.02	61.79[c]±1.33	80.56[d] ±1.59	52.07[e] ±0.89
Residual FAN (mg/L)	76.31[a]±1.46	31.64[b] ±1.25	91.72[c] ±2.07	89.16[e] ±1.65	13.87[d] ±1.57
Residual sucrose (g/L)	11.35[a] ±0.79	*ND	*ND	6.98[b] ±0.55	*ND
Residual glucose (g/L)	*ND	*ND	*ND	*ND	*ND
Residual fructose (g/L)	*ND	*ND	*ND	*ND	*ND

Ethanol yields, residual FAN and sugars from fermented stalk juices of SSV2, KSV8 and KSV3 sorghums cultivated in sites B and Z. Results were mean of triplicates. Means on the same row that do not share same superscript letter (a-e) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test. *ND = Not Detected.

To further improve on the fermentation performance of SSV2Z and SSV2B juice substrates i.e. to enhance the level of sucrose utilisation, the juice nutrient was further enriched by supplementing with a proportion of SSV3B juice that had higher amino acids and FAN concentrations than both SSV2Z and SSV2B (Table 2.4 and Fig. 2.3). Furthermore, SSV2Z juice was supplemented with SSV2B juice, because the SSV2B contained higher total sugars, Group 2 and "Other Group" amino acids than KSV3B juice (Tables 2.3 and 2.4).

2.2.3. Fermentation performance of juice blends.

Subsequent to the enrichment of SSV2Z nutrient content, three batches of hydrolysed juice blends were obtained i.e. (SSV2Z+KSV3B), (SSV2Z+SSV2B) and (SSV2B+KSV3B) respectively. Due to further hydrolysation of the juice blends with Promalt 295 (Kerry Bioscience). Juice soluble starch and protein polymers were further degraded to sugars and smaller protein polymers/molecules. Therefore, data summarised in Table 2.6 indicated that initial total sugars and FAN concentrations of SSV2Z raw juice were improved by about 10% after blending with KSV3B and ~7% after blending with SSV2B. Equally, the SSV2B initial total sugars and FAN were improved by over 5% after blending with KSV3B raw juice. However, it is expedient to mention that the final sucrose contents for all the juice blends were lower than

those of the corresponding initial raw juices. This was because due to juice blends hydrolysis, activities of latent endogenous *invertase* in splitting of sucrose molecules to glucose and fructose may have significantly increased, while sucrose content of hydrolysed juice reduces, the glucose and fructose contents increases, the increase in glucose content may also be attributed to activities of supplemented *amylases* (Promalt^{TM} 295) to degrade starch to glucose while the supplemented *protease* further hydrolysed crude proteins liberating more FAN into juices (Bitzer and Fox, 2000; Collar and Martinez, 2006; Fadlallah *et. al*, 2010; Davila-Gomez *et. al.,* 2011). Consequently, glucose, fructose and FAN concentrations of the juice blends were notably improved over the initial crude juice concentrations (Table 2.6).

Table 2.6 Initial sugars and FAN concentrations of SSV2 and KSV3 stalk juice blends

Item	**(SSV2Z + KSV3B)**	**(SSV2B + KSV3B)**	**(SSV2Z + SSV2B)**
FAN (mg/L)	353.56^{a} ±2.17	338.89^{b} ±1.96	342.79^{c} ±1.67
Sucrose (g/L)	98.64^{a} ±2.65	80.74^{b} ±1.88	101.87^{a} ±2.03
Glucose (g/L)	51.45^{b} ±1.11	56.67^{c} ±2.05	48.41^{b} ±1.94
Fructose (g/L)	27.39^{a} ±0.57	24.70^{b} ±1.02	23.35^{b} ±1.14
Total sugars (g/L)	177.48^{a} ±2.36	162.11^{b} ±3.72	173.63^{c} ±3.79

Two aliquots of SSV2Z sorghum raw juices (70 mL) were each supplemented with SSV2B and KSV3B raw juices (30 mL) respectively while SSV2B raw juice (70 mL) was also supplemented with KSV3B juice (30 mL), the final juice blends were hydrolyzed with exogenous enzymes supplementation by incubation at 70^{o}C for 1 h. SSV2 and KSV3B sorghums were cultivated in Kano (Sites B) and Kaduna (Site Z), Nigeria. Means on the same row that do not share same superscript letter (a-c) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

The ethanol yield potential of SSV2Z raw juice was improved by about 15% and 12% when it was pre-treated with raw juice from KSV3B and SSV2B respectively. Furthermore, the SSV2B showed an improved ethanol yield of about 17% when its raw juice was pretreated with raw juice from KSV3B (Fig. 2.5). The observed ethanol yields of 95 g/L and 92 g/L for (SSV2Z+KSV3B) and (SSV2Z+SSV2B) juice blends were similar to the ethanol yield of 98 g/L reported by Ariyajaroenwong *et al.* (2012) for sugar re-concentrated sorghum stalk juice (230 g/L total sugars) and fermented

by *S. cerevisiae* for 72 h with immobilization of yeast cells on sorghum stalks. However, both the 95 g/L and 92 g/L observed ethanol yields reported in this study are higher than the 86 g/L ethanol yields reported by Zhao *et al.* (2012) after supplementation of sorghum stalk juice with Urea/KH_2PO_4/$MgSO_4$ and fermented by *S. cerevisiae* yeast for 72 h.

However, Sridee *et al.* (2011) reported an ethanol yield of 109 g/L for sorghum stalk juice re-concentrated to about 280 g/L total sugars with cane molasses as adjunct and fermented at very high gravity (VHG) with exogenous nitrogen supplementation using dried spent yeast (DSY). This result was similar to results reported by Laopaiboon and Laopaiboon (2012), they reported ethanol yield of 109 g/L for sorghum stalk juice re-concentrated to 277 g/L total sugars and fermented under VHG with nitrogen supplementation using peptone/yeast extract/ammonium sulphate. An even higher ethanol yield of 121 g/L was reported further by Laopaiboon and Laopaiboon (2012) when cane sucrose was used as adjunct to re-concentrate sorghum stalk juice to 280 g/L total sugars and fermented under VHG with peptone/yeast extract/diammonium phosphate supplements as nitrogen sources. All these results were improvements over the reported findings in this study and suggested ways of improving SSV2 ethanol yields through enrichment of the juice substrate with commercially available nitrogen sources as well as employing improved fermentation techniques. However, in terms of low production cost, our findings appeared to be a cost-effective approach with efficient fermentation performance. Table 2.7 suggested that the limiting factor of the fermentation processes were sugars i.e. no residual sugars were detected in fermentation broth but only residual FAN.

Table 2.7 Residual sugars/FAN for SSV2Z hydrolysed stalk juice blends

Item	(SSV2Z + KSV3B)	(SSV2B + KSV3B)	(SSV2Z + SSV2B)
Ethanol yield (g/L)	94.76[a] ±2.13	75.47[b] ±1.89	91.71[c] ±1.84
FAN (mg/L)	101.31[a] ±2.44	88.74[b] ±2.15	96.55[c] ±2.12
Glucose (g/L)	*ND	*ND	*ND
Fructose (g/L)	*ND	*ND	*ND
Total sugars (g/L)	*ND	*ND	*ND

Ethanol yields, residual FAN and sugars from fermented hydrolysed juice blends of SSV2 and KSV3 sorghums cultivated in sites B and Z. Results were mean of triplicates. Means on the same row that do not share same superscript letter (a-e) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected

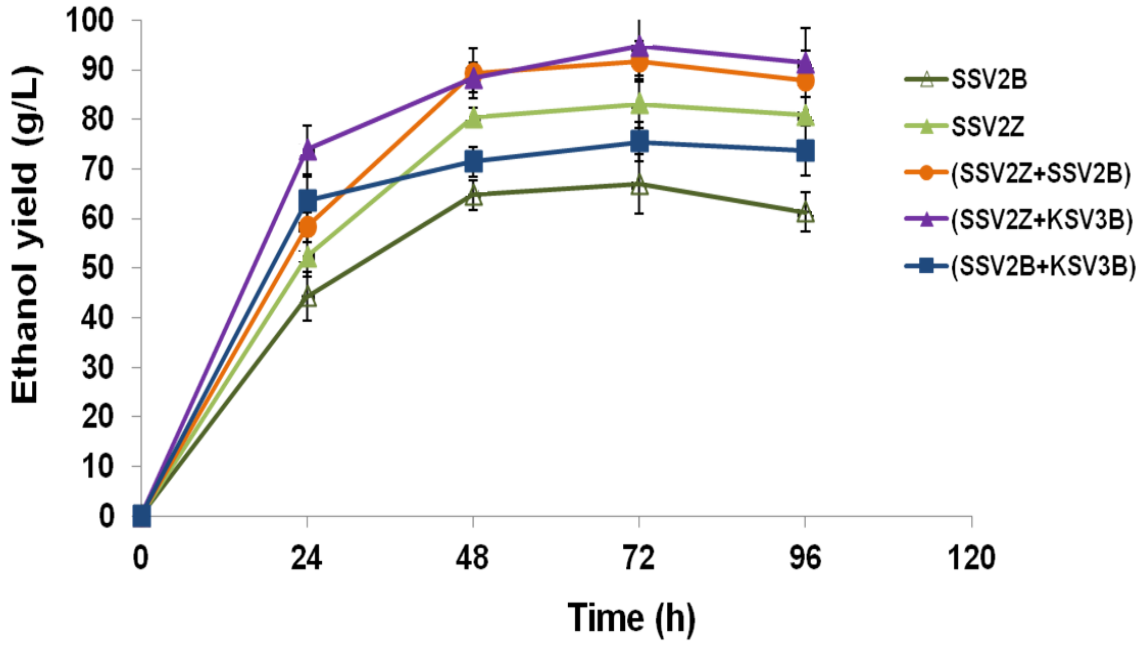


Fig. 2.5 Hydrolysed SSV2 juice blends fermented by *S. Cerevisiae.* The fermentation profile of SSV2Z juice supplemented with 30%v/v of SSV2B and KSV3B raw juices as well as that of SSV2B juice supplemented with 30%v/v KSV3B juice. 100ml of juice blends are inoculated with 1.0×10^7 cells/mL of *S. cerevisiae* and incubated at 32°C and 130 rpm. Ethanol yield were determined by GCMS-QP2010 equipment. Data are means of 2 independent replicates.

2.3 Conclusions and recommendations

These study findings suggested when cultivating a sorghum crop destined for stalk juice bioethanol production, a moderately wetter and colder climatic condition will favour improved stalk juice yield thereby resulting in corresponding improved bioethanol yield potential by over 20%. In addition to the crop cultivation site

selection, selecting the right and suitable sorghum cultivar may further increase ethanol yield potential by over 40%. Among the three Nigerian sorghum cultivars investigated in this study, SSV2 appeared the most suitable substrate with an estimated 3.963 t/ha fermentable raw juice sugar yield in Kaduna, this corresponded to 2,505 L/ha ethanol yield. The fermentable sugar yield potential for SSV2 raw juice in Kaduna was further improved to 4.355 t/ha (representing about 10% increase) by blending with KSV3 (Kaduna) raw juice followed by hydrolysis. This improved sugar yield corresponded to an increased ethanol yield of 2,947 L/ha (representing about 15% improvement).

Consequently, fermentable sugars and ethanol yields of SSV2, KSV8 and KSV3 sorghums may be further improved by cultivating crops under appropriately applied chemical fertilizer enriched soil. However, this might impact negatively on both the environment and overall bioethanol production cost if not prudently managed. For optimum sorghum juice ethanol production cost, enrichment of juice that is low in nitrogenous compounds with selected sorghum juice richer in nitrogenous compounds (but low in sugar content) may be a competitive and cost effective method of achieving optimum juice fermentation efficiency at low cost. Finally, this study showed that when cultivating sorghum for juice ethanol production, the appropriately chosen sorghum cultivar crops can be cultivated under wetter and colder conditions without necessarily applying fertilizer and/or irrigation water on moderately suitable soil quality. This will immensely help reduce competition for resources between energy crops and food crops.

CHAPTER THREE

Husked sorghum crude grains fermentation performance

3.1.0. Introduction.

Sorghum grains come in various shapes and sizes depending on cultivar type and crop growing conditions. Sorghum grain is the 2nd most important cereal in Africa and the 5th worldwide, it is cultivated on over 45 million hectares of farmland and annual global production output is estimated at over 60 million metric tonnes (Imam and Capareda, 2011; Zegada-Lizarazu *et. al.* 2012). Sorghum grain is a major source of staple food to over 500 million people residing in tropical regions of the world (Etuk *et al.,* 2012). The grains may be locally processed and consumed in Africa and Asia as Pap, Injera, Tuwo, porridge and so on. The grains are also sometimes used as adjunct in commercial beer brewing or malt drinks production typically in Africa (Fig. 3.1). In the USA, the grains have even wider commercial scale applications such as in pop corn production (Chopra, 2001; ICRISAT, 2004; FAOSTAT, 2011; USDA, 2012).

Fig. 3.1 An examples of sorghum grains-based beverages in Africa. Sorghum grains are used as adjunct in brewing and malting processes in Nigeria. Source; Taylor and Taylor (2007).

Sorghum is known for its adaptability to a wide range of climatic conditions and a high tolerance to various biotic and abiotic environmental stresses. The crop is known for its resistance to numerous crop diseases that commonly infect other cereal plants, for example, sorghum is not infected by *Peronosclerospora heteropogoni,* a fungus that causes downy mildew in maize with resultant loss of grain yields. Nevertheless, vast hectares of sorghum farmlands are continually being invaded by quelea birds in Africa and Asia with resultant massive loss of grains to farmers (Ofor *et al*., 2009; Ismail *et. al.*, 2010). Furthermore, despite the high resistance of sorghum crops to a range of common cereal crop diseases, fungal/bacterial diseases like grain smut, rust or mold among others constitutes major sources of sorghum grain losses to farmers either in the fields and/or in post-harvest storage facilities (USAID, 2009). In addition to these fungal and bacterial infections of sorghum grains, pests such as grasshoppers and rodents constitutes

another dimension of grain losses to farmers, especially where poor grain storage facilities abound, such as in rural areas of Africa/Asia (Ezeaku and Gupta, 2004; USAID, 2009; Yago *et. al*., 2011). Consequently, residual grains from disease infested farmlands, quelea birds or grasshoppers invaded fields and degraded grains from post harvest storage facilities (invaded by pests) are usually fed to livestock. These infested/defective grains are sometimes cleaned, blended with sound healthy grains and sold in the open market as sound grains. However, in spite of health concerns raised by experts concerning the use of fungal-infested or degraded sorghum grains as livestock feeds or a food source for humans, the practice appeared to progress through dubious farmers and their agents. This is largely because the farmers lack alternative economically rewarding means of disposing such degraded grains (Ismail *et. al.*, 2010; Etuk *et. al.*, 2012; Lyumugabe *et al*., 2012). It was envisaged in this study that, providing a commercially viable platform for utilisation of such degraded grains would not only minimise farmer's loss due to sorghum grains disease infections, but the platform would also help save humans and livestock from getting infected by such fungal diseases through utilisation of such spoilt grains as feed/food.

The fermentation performance of sound sorghum grains in malting and brewing processes has been extensively studied (Agu, *et. al*., 2006; Showemimo, 2007; Ijasan, *et. al.,* 2011; Ng'uni, *et. al.*, 2011). But very limited attention has been given to investigating the potential for bioconversion of spoilt or degraded sorghum grains to ethanol (Ismail *et. al.*, 2010; Yago *et. al*., 2011; Panchal and Dhale, 2011; Etuk *et. al.*, 2012). Bioethanol is an important liquid road transport fuel that may be used as an additive and/or substitute for gasoline in automobiles (Defra, 2008). Consequently, in this study, the potentials for bioconversion of whole crude sorghum

grains (comprising the husks, spikelet, awn, rachis and pubescence materials) to ethanol using various grain mashed batches as fermentation substrates was investigated. The outcome of the study aimed to provide a platform for commercial utilisation of residual sorghum grains from field or warehouse in bioethanol production. Three Nigerian sorghum grains known as SSV2, KSV8 and KSV3 were investigated in this study.

3.2.0. Study background

Nigeria, ranked the 2nd or 3rd largest sorghum grain producer in the world has over 9 million metric tonnes of sorghum grain production capacity annually (Nasidi *et al*., 2010). The Federal Government of Nigeria, through the federal ministry of agriculture is currently putting in place a comprehensive policy package to increase sorghum production output by minimum of 15% annually for 3 consecutive years, taking 2011 as the base year. The policy portfolio includes use of improved sorghum seedlings and introduction of mechanised farming technologies to local farmers. Therefore, creation of a wider market opportunity (such as increased commercial utilisation of the sorghum grains) is likely to attract more farmers to venture into sorghum production to exploit this potentially new economic benefit (FMARD, 2012). Currently, over 80% of annual sorghum grains produced in Nigeria is locally consumed directly as food or livestock feed while less than 5% is utilised in the brewing and malting industries (NAERLS, 2008; Hussaini *et al.,* 2009; Galadima *et al.,* 2011; Codex, 2012). Furthermore, additional 10% of the annual sorghum harvests are reported as field wastes due to pests or fungal invasions of farmland. Poor post-storage facilities are also a big source of grain loss, largely due to rodent attacks on the warehouse (NAERLS, 2008; Basavaraj *et. al*., 2012; Han, *et. al*., 2012). For example, a 2008 field survey reported by FMARD (Nigeria), estimated

that 13% of total sorghum grain produced in that year (2008) was lost to persistent quelea birds and pest invasion of farmlands. The USAID (2009) reported that an estimated 200,000 metric tonnes of post-harvested sorghum grains were lost annually to pests and fungal attacks on poor storage facilities in Nigeria.

In Nigeria, concerns of health risks regarding consumption of spoilt/degraded sorghum grains as feed or food have been raised and subsequently, relevant government agencies have been battling to completely end the use of degraded grains either as livestock feed or food. However, dubious farmers or their agents continue to "clean" and sell this degraded grains to unsuspecting public end-users (Chopra, 2001; Ameh *et. al.*, 2008; Ismail *et al.,* 2010). This practice appeared to continue because farmers (especially the peasant farmers) are keen to minimise their production loss by not disposing the spoilt grains without gaining economic benefit. For example, instead of burning degraded grains sourced from disease infested fields or pest invaded post-harvest storage facility, they rather pre-treat and sale such grains (Amusa and Falola, 2004; Bandyopadhyay, *et. al*., 2008; Akinyele, 2009; Panchal and Dhale, 2011). Consequently, it is envisaged in this study that degraded/spoilt sorghum grains may represent a sustainable feedstock source for the emerging bioethanol sector in Nigeria. Utilising spoilt grains for bioethanol production in Nigeria would offer farmers a viable and economically rewarding option for disposing degraded or spoilt sorghum grains.

It is expedient to mention that though the SSV2, KSV8 and KSV3 sorghum grains used in this study may not be categorically classified with certainty as spoilt and/or degraded residual grains from fields or post harvest storage facilities, some fractions of the grains were observed to show signs of mold infection and even after chemical

pre-treatment of the grains before planting, on harvesting, some of the crop's heads appeared infected with grain mold (Fig. 3.2); although the mold infection may arguably have originated from either the grain seedlings or from the field that might have been previously invaded by the grain mold fungus (Chopra, 2001; Ameh *et. al.*, 2008). The SSV2, KSV8 and KSV3 sorghum grains are widely cultivated in Nigeria, they are utilised both as food and as a feedstock source for the brewing industries. Particularly the SSV2 sorghum is most commonly cultivated for its sweet stalk juice that is used locally for sugary syrup production (Hussaini *et al.,* 2009).

Fig. 3.2 Pre-matured harvested KSV3 sorghum grain head.
KSV3 sorghum grain harvested 16 weeks after planting. The planted seedlings were from bulk samples used in this study. Early stage smut infestations were observed on grains.

3.3.0 Materials and Methods.

Nigerian local SSV2, KSV8 and KSV3 sorghum cultivar heads were obtained from the National Horticultural Research Institute (NIHORT, Nigeria). The crop heads were directly cut from field. The grains are manually removed along with husks, spikelet, awn, rachis and pubescence materials (Fig. 3.3). The crude husked grains were hammer milled (Ohaus®, Switzerland) and the final crude flours packed into air-tight bags and stored at 4^{o}C until ready to use.

Fig. 3.3 Crude grains of SSV2, KSV8 and KSV3 sorghum cultivars.
Sorghum crude grains (comprised of husks, spikelet, awn, rachis and pubescence materials). **(a)-** Left: SSV2 grains. **(b)-** Middle: KSV8 grains. **(c)-** Right: KSV3 grains.

3.3.1 Grains physical parameters analysis.

Preliminary analyses conducted on the crude flours of SSV2, KSV8 and KSV3 sorghums included moisture and ash content analysis, these were determined in accordance to NREL's standard laboratory analytical procedure (Hames *et. al.,* 2008). The thousand-kernel weight analysis was done by randomly selecting 1000 good-grade and de-husked kernels each of SSV2, KSV8 and KSV3 sorghums. The cleaned and pearled grains (1000 kernels) from each sorghum cultivar were weighed using a mass balance (Dykes and Rooney, 2006; McDonough, *et. al.*, 2008). The mean of each triplicate weight was finally recorded.

Furthermore, additional 500 pearled kernels each of SSV2, KSV8 and KSV3 sorghums were selected to determine condensed tannin content by "quick bleach method" as follows; bleaching reagent was prepared by dissolving anhydrous NaOH pellets (5 g) into 100 mL commercial JIK™ bleach (Reckitt Benckiser, UK) and allowing the solution to settle at room temperature for 15 min. The randomly selected

sound grains (100 kernels) each of SSV2, KSV8 and KSV3 sorghum were each placed in separate empty 50 mL glass beakers, afterwards, the bleaching reagent was added into beakers (just sufficient to completely cover whole grains surfaces). The beakers were covered with foil paper and incubated at 30^{o}C for 25 min in water bath with orbital shaking at 100 rpm. Finally, the bleached grains were thoroughly washed with water followed by gentle blotting with paper towels and finally allowed to dry at room temperature. The condensed tannin bearing grains were expected to reveal black/dark spots after the bleaching process by this test as shown in Fig. 3.4. Condensed tannins are normally present in the testa layer of sorghum grains; therefore, by bleaching or removing the grain outer coat layers, the testa is exposed. If the grain contains tannins, the testa appeared with dark or black spots and if otherwise it appeared white or retained its original grain colour (Waniska, *et. al.*, 1992; Dykes and Rooney, 2006; McDonough, *et. al.*, 2008).

Fig. 3.4 Example of sorghum grains tannin bleached test.
Source; Taylor and Taylor (2007): Normal unbleached sorghum grains (top row), and corresponding bleached grains (lower row); Note lower grains are partly or wholly covered with black spots; indicating presence of tannin in grains.

3.3.2 Crude grains compositional analysis.

The physico-chemical properties of the crude floured grain samples of SSV2, KSV8 and KSV3 sorghums were analysed. Proteins, lignin, total starch and pasting properties are the properties determined as briefly described below;

i. Proteins determination.

Protein fractions comprising albumins, globulins, glutelins and kafirins were sequentially determined by modified Osborne-Mendel method (Fig. 3.5) while the total crude proteins were determined following digestion of crude floured samples with NaOH solution (Youssef, 1998; de Mesa-Stonestreet *et. al.*, 2010). The difference between the total crude protein results obtained and the sums of the cumulative albumins, globulins, glutelins and kafirins protein fraction results represented what was considered "residual proteins" in this study, i.e.

Residual proteins = Total crude proteins - Σ(albumins, globulins, glutelins, kafirins).

a. Albumins, globulins, glutelins and kafirins proteins determination:

Floured samples (2 g dry wt.) each of SSV2, KSV8 and KSV3 sorghum crude grains were separately added into Erlenmeyer conical flasks containing 30 mL distilled water, the mixtures were manually swirled for 2 min at room temperature followed by incubation in a rotating shaker at 120 rpm and 65^{o}C for 90 min. The resultant slurry was centrifuged at 3800 rpm for 10 min and the supernatant (containing solubilised albumin) was decanted into fresh flasks and refrigerated, the bottom residues in each flask were washed by re-suspension in distilled water followed by centrifuging to remove the wash water and the final residue retained in original conical flask for globulin extraction.

Thus, 0.5M NaCl solution (30 mL) was added into conical flasks containing the retained washed bottom residues (from above) and the mixture stirred at room temperature for 2 min followed by incubation in a rotating shaker at 120 rpm and 65^{o}C for 90 min. The final slurry was centrifuged at 3800 rpm for 10 min, the supernatant (containing solubilised globulins) was decanted into fresh flasks for globulin concentration determination whilst the bottom residues were retained in original flasks. The previous procedures were repeated sequentially by adding 0.1M NaOH solution (30 mL) and 70%v/v ethanol solution (30 mL) to successive bottom residues sequentially to determine glutelins and kafirins protein concentrations in the supernatants respectively (Fig. 3.5). The albumins, globulins, glutelins and kafirins protein concentrations in supernatants were determined by Bradford™ Coomassie reagent (Sigma-Aldrich, UK) in accordance to manufacturer's protocol.

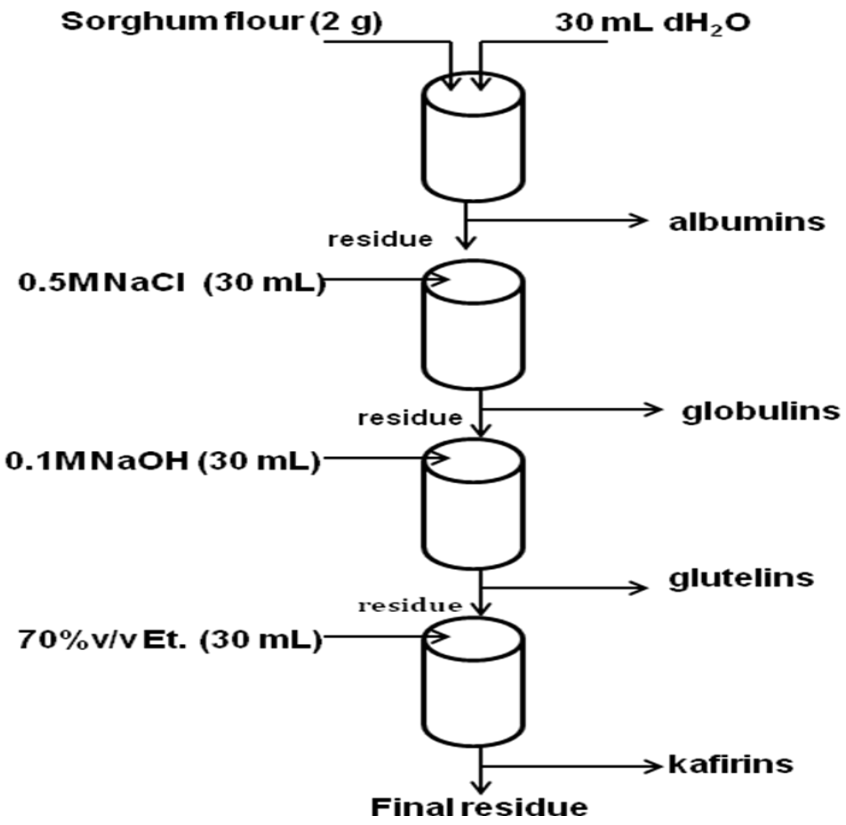


Fig. 3.5 Protein fractions extraction processes. Schematic diagram of Osborne-Mendeliv method for protein fraction extraction as used in this thesis.

b. Crude proteins determination

Floured samples (2 g dry wt. each) of SSV2, KSV8 and KSV3 sorghum crude grains were added into 2M NaOH solution (50 mL), the mixtures were stirred at room temperature for about a minute followed by incubation in a rotary shaker at 120 rpm and 60^{o}C for 2 h. The final mixtures were centrifuged at 3800 rpm for 10 min, the supernatant solution (which contains the solubilised crude proteins) were decanted and the protein concentrations determined by Bradford™ Coomassie reagent (Sigma-Aldrich, UK) in accordance to manufacturer's protocol.

ii. Total lignin: the total lignin comprise acid soluble and acid insoluble lignin (i.e. total lignin = Acid Soluble Lignin + Acid Insoluble Lignin), this was determined by modified Aldaeus and Sjöholm (2011) method.

a. Acid soluble lignin; the floured samples (1 g dry wt. each) of SSV2, KSV8 and KSV3 sorghum crude grains were added into conical flasks containing 72%v/v H_2SO_4 acid solution (10 mL), the slurries were incubated in a rotating shaker at 120 rpm and 30^{o}C for 45 min. Afterwards, 250 mL distilled water was added into the resultant mixtures and further incubated in a rotating shaker at 120 rpm and 65^{o}C for 2 h. The final mixtures were centrifuged at 3800 rpm for 10 min and the supernatants (containing the acid soluble lignin i.e. ASL) were collected into fresh glass beakers. Aliquots (2 mL) of the supernatant solutions were transferred into quartz cuvettes and the absorbance read at 205 nm with Genesys™ spectrophotometer (Thermo Spectronic®, USA).

b. The retained bottom residues (from above) were oven-dried at 60^{o}C for 48 h and the weights measured as the acid insoluble lignin (AIL).

c. The total lignin was evaluated as summation of acid soluble lignin (ASL) and acid insoluble lignin (AIL) i.e. (Total lignin = ASL + AIL)

iii. Total starch determination: the total starch content of the crude sorghum flours were determined by Megazyme™ K-TSTA standard protocol using modified AACC method 76.13 (Zhang and Hamaker, 1998).

Starch pasting properties: Paste viscosity is a function of swollen starch granule's ability to resist irreversible deformation during heating with constant stirring (i.e. beyond the starch granules gelatinization temperature range). Therefore, while paste peak viscosity indicates ability of starch granules to freely swell up to a point of physical breakdown, the paste final viscosity indicates the total breakdown of starch granules ability to form gels after cooling the paste to about 50^{o}C, see Fig. 3.6 (Sandhu and Singh, 2007; Sang, *et. al.,* 2008; Wireko-Manu *et al*, 2011). The starch pasting properties of SSV2, KSV8 and KSV3 sorghum flours were determined by RVA-4™ Rapid Visco Analyzer (Newport Scientific, Australia) using modified Newport scientific method ST-00 in accordance to the Scotch Whisky Research Institute Edinburgh (SWRI) standard procedures. An example of the methodology for KSV8 flour is:

1- Pre-dermined moisture content (MC) of flour = 9.86% (Table 3.4).

2- Therefore, dry matter (DM) content = (100 - MC) = 90.14%.

3- Standard equation: required sample weight (S) = (3.0 × 86.0)/DM, where 3.0 and 86.0 are constants (SWRI, Edinburgh). Hence, S = (3.0 × 86.0)/90.14 = 2.86 g flour.

4- Weight of mixing water required W = (28.0 - S) = (28.0 - 2.86) = 25.14 g dH_2O.

Briefly, 2.86 g of flour was added into canister containing 25.14 g water. The suspension was homogenised by properly stirring with glass rod at room

temperature. A paddle was placed into the canister and afterwards inserted into the Rapid Visco-Analyser for analysis. The instrument was switched on and allowed to pre-heat to 50^{o}C. Total analysis cycle time is 15 min. Typical RVA cycle profile is provided in Table 3.1.

Table 3.1 The RVA run temperature profile

Cycle time profile.	Parameter.	Value.
00:00:00	Temperature.	50^{o}C
00:00:00	Speed	960 rpm
00:00:10	Speed	160 rpm
00:00:30	Temperature.	50^{o}C
00:04:30	Temperature.	98^{o}C
00:09:00	Temperature.	98^{o}C
00:11:00	Temperature.	65^{o}C
00:15:00	Temperature.	65^{o}C

Note: idle temp. = 50^{o}C, total cycle time = 15 min, readings interval = 4 s.

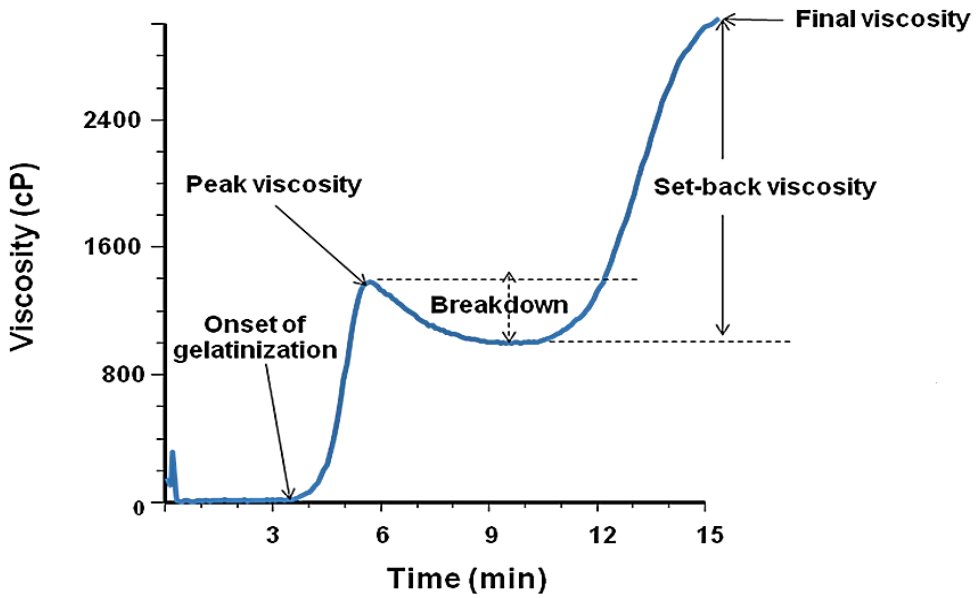


Fig. 3.6 Typical sorghum flour pasting property viscogram. Typical Rapid Visco Analyser (RVA) viscogram profile.

3.3.3 Starch mashing.

Mashing is an important step for successful starch fermentation, it is the process that activates and allows hydrolytic enzymes to degrade carbohydrates and complex

protein polymers to simple sugars and smaller protein molecules. These hydrolytic enzymes play specific roles in the liberation of fermentable sugars and yeast assimilable nitrogen compounds in worts during mashing (Goldammer, 2008; Wong *et al.*, 2009). Un-malted sorghum starch is considered "poor" in latent enzyme activity (Zhang and Hamaker, 1998; Owuama, 1999; Agu *et al.,* 2012; Chiba, *et al.*, 2012). However, for the purpose of this study, SSV2, KSV8 and KSV3 crude sorghum flours were un-malted so as to possibly reduce the over-all processing time and cost for spoilt grains ethanol. Four separate mashing substrates, comprising each of SSV2, KSV8 and KSV3 sorghums were prepared for mashing with corrosponding enzyme cocktails shown in Table 3.2. It is expedient to further mention that unlike in the conventional mashing process for sorghum grains whereby the grains were initially steeped in water and malted prior to mashing (Agu *et al.,* 2012; Chiba, *et al.*, 2012), in this study, the crude grain samples were directly prepared for mashing without prior steeping or malting due to inclusion of husks, awn, rachis and pubescence grain materials. This in part, is to investigate the potentials for direct bioconversion of degraded/spoilt sorghum grains to ethanol using low cost methods and without necessarily investing further resources on pre-treatment of the degraded grains prior to mashing.

Table 3.2 Composition of exogenous hydrolytic enzymes

Notation	Enzyme	Activity	Description
E1	Promalt™ 295	500BG[a] µ/mL-min	*β-glucanase/β-amylase/protease*
E2	Bioglucanase™ ME1250L	750BG[a] µ/mL-min	*α-glucanase*
E3	Hitempase™ 2XL	4416 µ/mL-min	*α-amylase/β-glucanase*
E4	Promalt™ 4TR	300BG[a] µ/mL-min	*β-glucanse/β-amylase/protease*
E5	Termamyl® 120L	120 KNU[b]/g	*α,β-amylase*

Enzymes generously provided by Kerry Biosciences, Northern Ireland.
[a]betaglucanase unit/mL
[b]Kilo Novo α-amylase Units (KNU)

Floured crude grain samples (30 g dry wt.) of SSV2, KSV8 and KSV3 sorghums were mixed with distilled water (70 mL) in conical flasks, the mixtures were manually stirred with glass rods for 2 min at room temperature. Afterwards, the mixtures were cooked in a water bath at 80^{o}C for 60 min with constant stirring. Furthermore, distilled water (50 mL) was added to the mixtures and autoclaved at 121^{o}C for 15 min. The final slurry samples (30% w/v) each of SSV2, KSV8 and KSV3 sorghum were further prepared into four batches (i.e. batch 1-4) and each batch comprise aliquots of SSV2, KSV8 and KSV3 mash samples. Table 3.3 summarised data of enzyme cocktails employed for mash batches preparation.

Table 3.3 Mash batches and exogenous enzymes cocktail

Batch	Enzyme cocktail	Dosage	Substrates
Batch-1	E1	150 µL	SSV2, KSV8 & KSV3
Batch-2	(E1 + E2)	150 µL	SSV2, KSV8 & KSV3
Batch-3	(E1 +E2 + E3)	150 µL	SSV2, KSV8 & KSV3
Batch-4	(E1 +E2 + E3 +E4 + E5)	150 µL	SSV2, KSV8 & KSV3

E1 = Promalt™ 295, E2 = Bioglucanase™ ME1250L, E3 = Hitempase™ 2XL, E4 = Promalt™ 4TR, E5 = Termamyl® 120L

- ✓ **Batch-1:** Promalt™295 enzymes (150 µL) comprising betaglucanase/ bacterial-amylase/protease (Kerry Biosciences, Northern Ireland) were added to mashing substrates and mash final volume adjusted to 200 mL with distilled water.
- ✓ **Batch-2:** Enzyme cocktails (150 µL) comprising equal volumes of Promalt™295 and Bioglucanase™ ME1250L (Kerry Biosciences, Northern Ireland) were added to mashing substrates and mash final volume adjusted to 200 mL with distilled water.
- ✓ **Batch-3:** Enzymes cocktail (150 µL) comprising equal volumes of Promalt™ 295, Bioglucanase™ ME1250L and Hitempase™ 2XL (betaglucanase /bacterial amylase), (Kerry Bio-sciences, Northern Ireland) were added to

mashing substrates and the mash final volumes adjusted to 200 mL with distilled water.

- ✓ **Batch-4:** Enzyme cocktail (150 µL) comprising of equal volumes of Promalt™295, Bioglucanase™ ME1250L, Hitempase™2XL, Promalt™4TR (bacterial betaglucanase/amylases/protease), (Kerry Bioscience, Ireland) and Termamyl™ 120L amylase (Novozymes, Denmark) were added to mashing substrates and the mash final volumes adjusted to 200 mL with distilled water.

Finally, four mashing substrate batches (in addition to control batch samples corresponding to each of the batch) were placed in a rotary shaking incubator and initially cooked at 50^{o}C for 120 min with 120 rpm orbital shaking, afterwards, further cooking at 60^{o}C for 60 min with 130 rpm orbital shaking. Test samples (1.0 mL) were withdrawn from each of the final worts, filtered through Luer-Lok™ micro syringe filters (Chromacol, USA) into 2.0 mL glass vials (Chromacol, USA), about 1.0 mL of HPLC calibrated internal standard sugar (Meso-Erythritol solution) was added to each vial. The vials were placed in HPLC autosampler (Spectra-physics, USA) and sugars separated with a 300 mm × 7.8 mm REZEX RPM-monosaccharide Pb+2 (8%) column^{TM} (Phenomenex, USA). The operating parameters for the HPLC are provided in Table 2.2 (pg. 48). Sugars were detected using refractive index and quantified using HPLC software (CSW32 version v.1.4 chromatogram software from DataApex®, USA). The total FAN concentrations of wort were determined by combination of K-LARGE kit to determine L-arginine/Urea/Ammonia (Rapid) and K-PANOPA kit to determine total Primary Amino Nitrogen (Megazyme, Ireland) according to the manufacturer's protocol.

3.3.4 Yeast seed culture preparation

Three sterile loopfuls of industrial strain *Saccharomyces cerevisiae (DCLM)* and *Pachysolen stipitis (NCYC1416)* yeast strains were each inoculated into a 400 mL YEPD media comprising 4.0%(w/v) bacteriological peptone, 2.0%(w/v) yeast extract and 4.0%(w/v) glucose. The YEPD cultures were incubated at 32^{O}C for about 20 h at 150 rpm in shaking incubators.

3.3.5 Starch mash fermentation

Fermentation progress was monitored in parallel by measuring cumulative CO_2 gas pressure formation using ANKOM^{TM} system (ANKOM^{RF} TECHNOLOGY, USA) and on the other hand using shaking flasks in incubator whereby samples are withdrawn every 24 h for alcohol yield determination by FermentoFlash® (Funke-Gerber^{TM}, Berlin).

1. ANKOM^{RF} system fermentation set-up

Filtered mashed worts (100 mL) from each mashing batch were added into 250 mL ANKOM^{RF} glass bottles followed by separate inoculation of 1.0×10^{7} cell/mL *S. cerevisiae* and *P. stipitis* yeasts cells respectively to each separate glass bottle. Fermentation was conducted in a rotary shaking incubator (at 130 rpm and 32^{o}C). The fermentation progress was monitored by automated measurement of cumulative CO_2 pressure formation after every 20 min via ANKOM^{RE} gas-production system (ANKOM Technology, USA) as shown in Fig. 3.7. Fermentations were allowed to run undisturbed until CO_2 gas production rates were observed to start declining. Van der Waals ideal gas law equation was employed to calculate equivalent CO_2 gas volume yield from pressure.

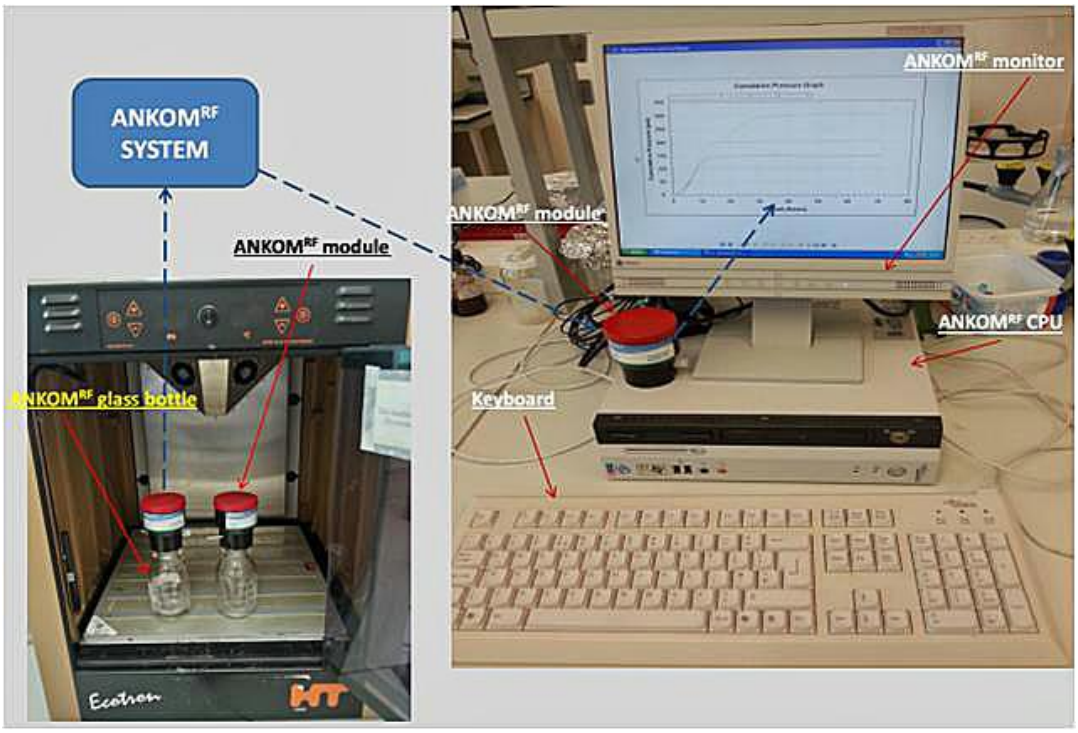


Fig. 3.7 Typical ANKOM^{RF} system set-up

Fermentation substrates are dispensed into ANKOM glass bottles, module cap screwed on bottles and placed in orbital shaking incubator, modules caps transmit CO_2 gas pressure signals to control module (adjacent to PC) which in turn transmit to ANKOM's CPU and the response translated on monitor graphically.

2. Shake-flask fermentation set-up

Filtered mashed worts (100 mL) from each mashing batch were added into 250 mL Erlenmeyer conical flasks followed by separate inoculation with 1.0×10^7 cell/mL of *S. cerevisiae* and *P. stipitis* into each flask. The flask tops were plugged with cotton wool and placed in a rotary shaker incubator set at 130 rpm and 32^{o}C. Samples were withdrawn after every 24 h for observed alcohol determination by FermentoFlash® equipment (Funke-Gerber^{TM}, Berlin). Fermentations were ended after 72 h runs.

Alcohol determination and CO_2 gas volume calculation

a. For alcohol concentration determination, about 11.0 mL of fermentation broth were added into 20.0 mL beaker, equipment's alcohol probe was dipped into the beaker and about 10.0 mL of the broth was sucked into the measuring cells. Finally,

alcohol concentration and density results were automatically printed via a printer integrated with the FermentoFlash® equipment.

The FermentoFlash® measurement principle:

The fermentation broth sample (10 mL) is sucked into the measuring cells by means of a pump. The alcoholic content and density of the fermentation broth are measured by using thermal measuring effects. Derived constituents as original wort, apparent extract and osmotic pressure are also determined but not reported in this sudy. The device is pre-calibrated with acqeous alcohol.

b. Total CO_2 gas volume produced by fermentation was evaluated from the measured cumulative CO_2 gas pressure by ANKOM^{RF} system. The peak cumulative CO_2 gas pressure were recorded along with the corresponding temperature, Van der Waals gas law equation (i.e. PV = nRT) was used to calculate the equivalent CO_2 gas volume, typical calculation example is shown below.

Typical calculation sample for CO_2 gas volume from ANKOM^{RF} measured CO_2 gas pressure (e.g. KSV8 mash sample);

- Cumulative measured CO_2 gas pressure (P) = 110.28 psi = 760.347 kPa.
- Corresponding measured temperature (T) = 32.4^{O}C = 305.4^{o}K.
- Glass bottle rated volume = 250 mL; Actual volume = 310 mL (ANKOM Tech).
- Fermentation wort Vol. = 100 mL.
- Head-space volume in glass bottle (V) = 310 mL - 100 mL = 210 mL (0.21 litres).
- Gas constant (R) = 8.314472 L·kPa$^{\cdot O}$K^{-1}·mol^{-1}
- Number of gas moles (n) = P (V/RT)-- Van der waals equation.

  Thus, n = 0.06288 mol.
- From Avogadro's Law; 1 mole gas occupies 22.4 litre Volume.
- Hence, cumulative CO_2 gas produced (mL) per 100 mL of wort = 0.06288 mol × 22.4 L/mol × 1000 mL/L = 1409 mL/15 g dry flour or 93.90 kL/t dry flour.

3.3.6 Statistics analytical method

Significant difference between means was tested by ANOVA using Turkey method by Minitab™ 16 statistical software (MINITAB©, USA). Means that do not share a superscript letter (a-f) within same rows are significantly different ($p \leq 0.05$) based on grouping information using Tukey method at 95% simultaneous confidence interval.

3.4.0 Results and discussions

The colour appearance of sorghum grain occasionally influenced its end-uses. For example, brown or red coloured grains are usually considered not suitable for certain snacks and beverage preparation because they impact their original grain colour on appearance of the final product (Awika and Rooney, 2004). Therefore, white sorghum grains are commonly preferred for malting and brewing operations in Nigeria partly because they usually contain no tannins and their white colour does not interfere with the final colour of the brew (USAID, 2009; Ogbonna, 2011). Consequently, KSV8 sorghum (white grain) has a wider potential industrial application than KSV3 and SSV2 that are both brown coloured grains (Ogbonna, 2011: USAID, 2012). Furthermore, KSV8 being white coloured grain also has wider local domestic consumption than both SSV2 and KSV3 grains that are relativey brown coloured, Fig. 3.8 (USAID, 2009). Furthermore, SSV2, KSV8 and KSV3 sorghum grains appeared tannin-free; no significant black/dark spots were visibly detected on the grain surfaces after the quick bleach (Fig. 3.9). The black dots noticeable on the germ portion of grains after the bleach test is normal for all sorghum grains (Taylor *et al.,* 2007).

Fig. 3.8: Pearled and floured KSV3, SSV2 and KSV8 sorghum grains.
Top row: de-husked and pearled grains of KSV3, SSV2 and KSV8 sorghums.
Bottom row: floured crude grains of KSV3, SSV2 and KSV8 sorghum.

3.9: Example of KSV3 and KSV8 sorghum grains tannin test results.
Top left: Unbleached KSV3 pearled sorghum grains. **Top right:** corresponding KSV3 pearled grains after bleaching with JIK™ reagent (Reckitt Benckiser, UK). **Bottom left:** KSV8 pearled grains. **Bottom right:** corresponding KSV8 bleached pearled grains.

3.4.1 Compositional analysis results

In addition to observed physical differences in colours and shapes of SSV2, KSV8 and KSV3 sorghum grains, results in Table 3.4 indicated that the chemical compositions of the grains are significantly different ($p < 0.05$) as well. For example, the starch content of KSV3 grains is significantly higher ($p \leq 0.05$) than that of SSV2 and KSV8 grains. Hence, this tended to suggest KSV3 will be more attractive as staple food source than KSV8 and SSV2. Furthermore, SSV2 grains having significantly higher lignin and ash content ($p \leq 0.05$) tended to suggest higher

phytates and minerals than both KSV3 and KSV8 grains (Waniska, 2000; Léder, 2004).

Finally, people with concerns on their gluten intake in food e.g. Celiac patients (Ciacci, *et al,* 2007), will tend to prefer consuming KSV8 flour with significantly ($p \leq 0.05$) lower gluten content as compared to SSV2 and KSV3.

1. Proteins and Free amino Nitrogen (FAN).

Proteins are important nitrogenous compound sources for yeast assmilable nutrients. For example, ammonium ions, amino acids and small peptides (e.g. di- and tri-peptides) are nitrogenous compounds that are important during fermentation for efficient yeast metabolism which includes healthy cell growth, cell osmotic pressure regulations among other functions (Lodolo *et al.*, 2008; du Plessis, 2008; Wong *et al*., 2009). Albumin and globulin proteins are more amenable to proteolysis followed by glutelins and kafirins (Osman *et al.*, 2001; Goldammer, 2008; Wong, *et al*., 2009). The albumins and globulins are more readily soluble during cooking compared to kafirins and glutelins, which are encapsulated in a complex starch-protein structure that is re-enforced by strong di-sulfide bonds. Thus, under light mashing or cooking conditions, the complex starch-protein matrix fails to completely dissolve and expose the bulk of the storage protein bodies (Kafirins and glutelins) and complex starch polymers to *proteolytic* enzymes activities (de Mesa-Stonestreet, 2010; Afify *et al.,* 2012; Serna-Saldìvar *et. al.*, 2012).

Table 3.4 Physico-chemical composition of SSV2, KSV8 and KSV3 sorghum flours

Parameter	SSV2	KSV8	KSV3
1000 kernel wt. (g)	$26.11^{a} \pm 2.54$	$30.40^{b} \pm 1.87$	$32.85^{b} \pm 2.63$
Moisture content (%)	$7.21^{a} \pm 0.55$	$9.86^{b} \pm 0.34$	$8.77^{a} \pm 0.41$
Ash content (%)	$2.81^{a} \pm 0.10$	$1.31^{b} \pm 0.07$	$2.25^{c} \pm 0.09$
Total lignin (g/100g flour)	$13.10^{a} \pm 0.13$	$10.58^{c} \pm 0.46$	$11.96^{b} \pm 0.51$
Total starch (g/100g flour)	$65.64^{a} \pm 1.67$	$69.87^{b} \pm 1.34$	$73.42^{c} \pm 1.86$
Total proteins (g/100g flour)	**$15.57^{a} \pm 0.79$**	**$14.31^{a} \pm 0.88$**	**$16.38^{b} \pm 1.12$**
Albumins (g/100g flour)	$1.31^{a} \pm 0.74$	$1.99^{b} \pm 0.46$	$1.56^{b} \pm 0.17$
Globulins (g/100g flour)	$2.47^{a} \pm 0.31$	$3.50^{b} \pm 0.55$	$2.63^{a} \pm 0.67$
Glutelins (g/100 flour)	$2.78^{a} \pm 0.68$	$1.46^{b} \pm 0.39$	$2.70^{a} \pm 0.11$
Kafirins (g/100 flour)	$6.35^{a} \pm 0.53$	$5.08^{b} \pm 0.72$	$5.90^{b} \pm 0.52$
Residuals (g/100g flour)	$2.66^{a} \pm 0.87$	$2.29^{a} \pm 0.69$	$3.69^{b} \pm 0.88$

Compositional analysis of SSV2, KSV8 and KSV3 sorghum crude grains: **(a)-** total lignin determined by modified Round Robin protocol (Aldaeus and Sjöholm, 2011). **(b)-** Total starch by Megazymes kit (K-TSTA-100™) **(c)-** Proteins were extracted by modified Osborne-Mendelev method while total crude protein were extracted by lime digestion. Means on the same row that do not share same superscript letter (a-c) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

2. Starch pasting properties

Starch pasting property is dependent on temperature, moisture content and the degree of viscosity of paste which in turn is dependent on amylose/amylopectin ratio of the original starch substrate. Whilst amylose starch structure is characterised as linearly arranged helical crystalline polymer chains, amylopectin is a non-crystalline and branched chain polymer with relatively higher solubility in water (at room temperature) than amylose (Udachan, *et. al.*, 2012). Sorghum starches typically contain 20-30% amylose starch and 80-70% amylopectin (Lyumugabe *et al*., 2012). Therefore, high amylopectin starches are considered more favourable to enzymatic saccharification than high amylose starch, this is because the former provide a larger surface area and free-end surface for amylolytic enzyme attachment/attack than the latter. This is in addition to the higher degree of solubilisation in water of high-amylopectin starches than high amylose starch (Van Hung *et al.,* 2006; Yan *et al*., 2011).

From Fig. 3.10, KSV3 followed by KSV8 starches showed higher final and peak viscosities than SSV2 starch. Starch samples with higher final and peak viscosities are normally associated with having higher amylose contents relative to starches with lower corresponding values (Van Hung *et al.,* 2006). This is because amylose starch dissolve easily during cooking forming a gel-like colloid that rapidly increases the paste viscosity (representing peak viscosity) while during paste cooling phase, the amylose starch granules take a longer time for their molecules to re-align back into their crystalline structure compared to the amylopectin starch granules that are amorphous in structure thereby required less time to settle back into their amorphous structure during paste cooling (Mutters and Thompson, 2009). Therefore, KSV3 followed by KSV8 tends to contain higher amylose starch content than SSV2 starch substrates (Fig. 3.10). Furthermore, data in Table 3.5 indicated that SSV2, KSV8 and KSV3 starches exhibited similar pasting temperatures. However, whilst KSV8 and KSV3 starches have similar peak times, the SSV2 starch exhibited relatively higher peak pasting time. The observed longer pasting time of SSV2 starch substrate is likely due to interference of its high lignin content with the pasting results compared to KSV8 and KSV3 substrates that have relatively lower lignin.

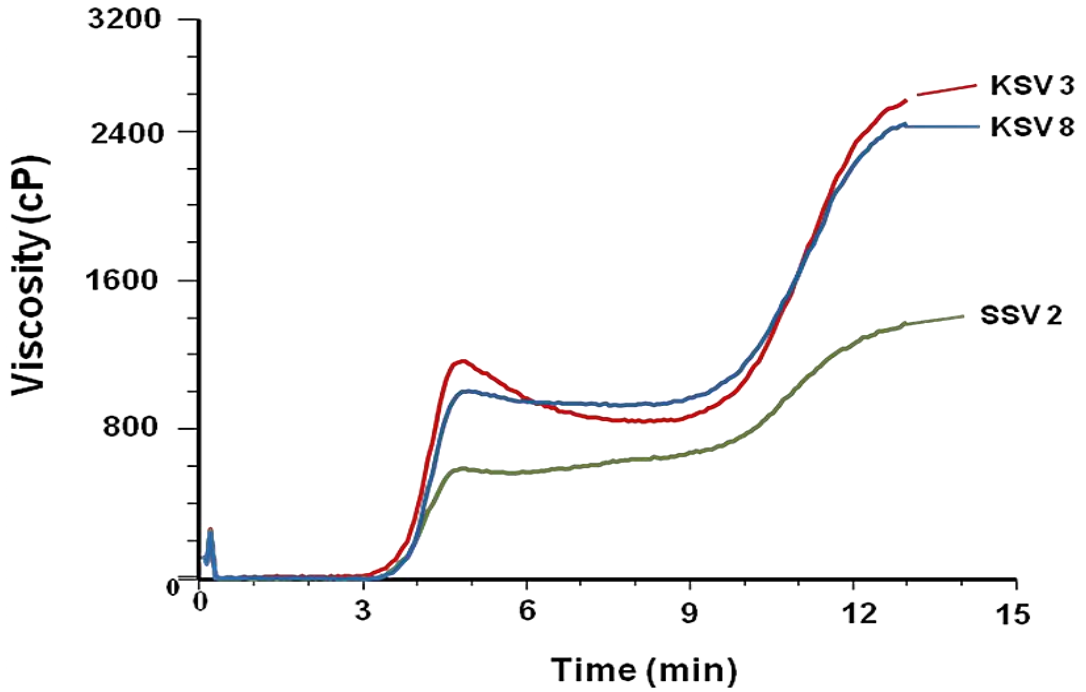


Fig. 3.10 SSV2, KSV8 and KSV3 floured sorghum crude grains pasting profile.
Pasting properties of SSV2, KSV8 and KSV3 sorghum crude flour (comprising grains, husks, awn, rachis and spikelet) determined by a rapid visco analyzer (model 3D+) in accordance with SWRI standard procedure. Table 3.1 provides the RVA temperature profile. Values are mean of 2 replicates runs.

Table 3.5 Pasting properties of SSV2, KSV8 and KSV3 sorghum flours

Cultivar	Peak viscosity (cP)	Set-back viscosity (cP)	Pasting Temp (°C)	Peak time (min)	Final viscosity (cP)
KSV3	$1167^{a} \pm 27$	$1729^{a} \pm 31$	$79.90^{a} \pm 1.0$	$4.80^{a} \pm 0.3$	$2569^{a} \pm 41$
KSV8	$1007^{b} \pm 40$	$1517^{b} \pm 19$	$81.45^{b} \pm 0.7$	$4.93^{a} \pm 0.5$	$2443^{b} \pm 33$
SSV2	$599^{c} \pm 21$	$804^{c} \pm 36$	$78.95^{a} \pm 0.8$	$6.87^{c} \pm 0.7$	$1372^{c} \pm 27$

Mean of 2 replicate experiments. Means in the same column that do not share same superscript letter (a-c) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

3. Starch mashing

Starch mashing is an important process of enriching fermentation wort with yeast nutrients. Carbohydrates and protein polymers are enzymatically hydrolysed to fermentable sugars and yeast assimilable nitrogen during mashing. Proteolytic enzymes (proteinases and peptidases) liberate simple amino acids and small peptides from complex protein polymers while amylolytic enzymes (α- and β-

amylases) degrades polysaccharides to simple monomeric and oligomeric fermentable sugars during starch mashing (Goldammer, 2008).

SSV2, KSV8 and KSV3 sorghum flours were initially mashed without exogenous enzyme supplementation to determine the efficiency of the endogenous hydrolytic enzymes activities of the substrate's during mashing (control samples). KSV8 control sample wort showed higher FAN levels while SSV2 wort showed higher fermentable sugars release (Table 3.6). These results were consistent with protein profiles summarised in Table 3.4, where KSV8 flour showed higher content of soluble albumins and globulins proteins (which are highly amenable to proteolysis) than SSV2 and KSV3 flours. Furthermore, in spite of SSV2 flour having the lowest starch content, it liberated higher fermentable sugars into wort during mashing compared to KSV8 and KSV3 worts that had a higher initial starch content (Tables 3.4 and 3.6). These results on one hand suggested that SSV2 substrate has higher latent active amylolytic enzymes activity than KSV8 and KSV3 substrates, and on the other hand tended to agree with pasting properties of the SSV2 substrate, which suggested it has higher amylopectin starch that is more amenable to enzymatic attack because amylopectin provides a larger surface area and free end for enzymes to attack (Van Hung *et al.,* 2006). Subsequently, the results of the four sample batches each of SSV2, KSV8 and KSV3 sorghum substrates supplemented with cocktail of enzymes were discussed below.

3.4.2 Batch-1: mashing with β-glucanase/β-amylase/protease

KSV8 wort shows relatively higher FAN and fermentable sugar concentrations and is followed by SSV2 and KSV3 worts (Table 3.6). These results indicated that the externally supplemented hydrolytic enzymes have improved carbohydrates and

proteins polymers degradation. Maltose sugar concentrations were observed to be about twice that of glucose sugars in the resultant worts. Hence, this suggested that the β-amylases are able to effeciciently breakdown the α-1-4 carbon bonds of amylose and amylopectin starches to liberate maltose. However, the action of β-glucanase were able to hydrolyzed beta-1,3 and beta-1,4 glycosidic bonds of β-Glucan to produce glucose (Suresh *et al.,* 1999). However, the concentration of xylose remained unchanged relative to the corresponding control samples because no exogenous hemicellulase enzymes were supplemented in the mashes (Gao *et al.,* 2011; Sukanya and Teeradakorn, 2011).

Table 3.6 Batch-1 mash and the corresponding control sample's liberated sugars and FAN concentrations

Parameter	SSV2	KSV8	KSV3	SSV2	KSV8	KSV3
	(Batch-1 exogenous enzymes*)			(control endogenous enzymes**)		
FAN (mg/L)	122.9^{a} ±2.45	133.42^{b} ± 3.02	118.39^{c} ±2.9	78.52^{d} ±1.8	88.35^{e} ±1.61	60.62^{f} ±0.99
Glucose (g/100g flour)	9.97^{a} ±0.87	12.46^{b} ±1.03	7.72^{c} ±0.65	4.91^{d} ±0.85	4.41^{d} ±0.53	3.56^{f} ±0.14
Maltose (g/100g flour)	18.26^{a} ±1.32	19.37^{a} ±2.17	15.31^{b} ±0.04	10.60^{c} ±1.1	10.10^{c} ±0.87	8.78^{d} ±0.69
Xylose (g/100g flour)	2.70^{a} ±0.30	2.99^{a} ±0.21	4.11^{b} ±0.16	2.56^{c} ±0.31	2.28^{c} ±0.66	2.93^{a} ±0.42
Total sugars (g/100g flour)	30.93^{a} ±1.37	34.82^{b} ±1.79	27.14^{c} ±1.48	18.06^{d} ±0.7	16.65^{e} ±0.83	15.27^{e} ±0.93

*exogenous enzymes mash comprised of commercially available betaglucanase, amylase and protease enzyme cocktails. **endogenous enzymes are latent originally present in flour samples. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test.

3.4.3 Batch-2: mashing with additional α,β-glucanase/β-amylase/protease

Further supplementation of the batch-2 mashes with α-glucanase (in addition to the initial batch-1 enzymes cocktail) led to increase hydrolysis of 1-6 carbon bonds of amylopectin to liberate more glucose molecules. Table 3.6 summarised observed

increase in glucose concentrations of batch-2 mashes (Zhang and Hamaker, 1998). The highest levels of fermentable sugars and FAN concentrations were found in KSV8 wort, followed by SSV2 and KSV3. Although no additional proteases were added to the mashes, it is conceivable that the improved degradation of carbohydrates lead to breakdown of the complex starch-protein granule matrices thereby freeing more proteins for proteolysis activities (Lyumugabe *et al*., 2012).

3.4.4 Batch-3: mashing with additional α,β-glucanase/α,β-amylase/protease

Further supplementation of batch-3 mashing substrates with thermostable α-glucanase (Hitempase^{TM} 2XL) showed notable increase in the levels of glucose and FAN in worts (Table 3.7). This could be associated with increase α-glucanases activities to further breakdown 1-6 carbon links of amylopectin to glucose. Previously study suggested that the higher the efficiency of starch hydrolysis, the higher the protein polymers that will be free from the starch-protein matrix for increased proteolytic activity (Zhang and Hamaker, 1998; de Mesa-stonestreet, 2008; Wong *et al*., 2009). Consequently, as more sugars are liberated in worts, more nitrogenous compounds are also liberated in wort. The KSV8 wort shows the highest level of total fermentable sugars and FAN concentrations followed by KSV3 and SSV2 worts respectively.

Table 3.7 Batches-2 and 3 mashes liberated sugars and FAN concentrations

Parameter	SSV2	KSV8	KSV3	SSV2	KSV8	KSV3
	(Batch-2 exogenous enzymes**)			(Batch-3 exogenous enzymes**)		
FAN (mg/L)	143.29^{a} ±2.7	151.87^{b} ±3.1	146.14^{c} ±2.5	147.28^{d} ±1.8	159.09^{e} ±1.9	154.20^{f} ±2.1
Glucose (g/100g flour)	20.30^{a} ±0.87	21.17^{a} ±1.03	14.52^{b} ±0.65	22.20^{a} ±1.16	27.66^{c} ±1.35	20.39^{a} ±1.04
Maltose (g/100g flour)	12.29^{a} ±1.24	15.83^{b} ±2.07	13.59^{a} ±1.31	18.89^{c} ±1.23	21.06^{d} ±1.07	23.20^{e} ±2.01
Xylose (g/100g flour)	2.86^{a} ±0.31	3.16^{b} ±0.22	4.89^{c} ±0.14	3.38^{b} ±0.41	3.75^{b} ±0.25	5.19^{c} ±0.38
Total sugars (g/100g flour)	35.45^{a} ±2.29	40.16^{b} ±1.19	33.01^{a} ±2.02	44.46^{c} ±2.55	52.47^{d} ±1.40	48.78^{e} ±2.44

**Batch-2 & -3 mashes exogenous enzymes comprised of betaglucanase, (α,β)-amylases, higher activity bioglucanase and protease. Means on the same row that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test.

3.4.5 Batch-4: mashing with additional α,β-glucanase/α,β-amylase/protease

Finally, the additional supplementation of Batch-4 mashes with combination of thermostable endoproteases (Promalt^{TM} 4TR) and thermostable α,β-amylase enzymes (Termamyl® amylase) lead to notable improvement in total fermentable sugars and FAN concentrations in worts (Table 3.8). The additional thermostable endoproteases in the mash increased proteolysis activities which resulted possibly in further degradation of glutelins and kafirins proteins that are not commonly hydrolysed by proteinase enzymes (Osman *et al*., 2001). However, no significant changes in concentration levels of xylose sugar in worts were observed possibly because no exogenous hemicellulase enzymes were added during mashing. In overall, batch-4 worts yielded the highest fermentable nutrient levels in worts.

Table 3.8 Batch- 4 mashes liberated sugars and FAN concentration

Parameter	SSV2	KSV8	KSV3
	(Batch-4 exogenous enzymes*)		
FAN (mg/L)	159.35[a] ±1.88	173.34[b] ±1.25	167.29[c] ±1.41
Glucose (g/100g flour)	24.22[a] ± 1.77	33.34[b] ± 1.13	34.07[b] ± 0.86
Maltose (g/100g flour)	33.63[a] ± 2.03	32.75[a] ± 1.89	29.19[b] ± 1.11
Xylose (g/100g flour)	3.46[a] ± 0.41	3.67[a] ± 0.24	5.48[b] ± 0.72
Total sugars (g/100g)	61.31[a] ± 1.31	69.76[b] ±1.40	68.74[b] ± 2.45

*****Batch-4 exogenous enzymes mash comprised of commercially available betaglucanase, protease and thermal stable (α,β)-amylases and (α,β)-glucanase enzyme cocktails. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-c) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

3.5 SSV2, KSV8 and KSV3 sorghum starch mashes fermentation

Fermentation performances of up to 10 different yeast strains on SSV2, KSV8 and KSV3 wort substrates were initially investigated during the preliminary design phase of this study. Industrial strains of *Saccharomyces cerevisiae* (DCLM) and *Pichia stipitis* (NCYC 1416) yeasts were consistently observed to show favourable ethanol and CO_2 gas yields relative to other strains of *S. cerevisiae*, *P. tannophilus, K. maxianus* among others (results not reported here). Therefore, *S. cerevisiae* and *P. stipitis* industrial yeast strains were chosen for the purpose of this study. The *S. cerevisiae* cells are reported to be efficient glucose fermenting yeast while *P. stipitis* are efficient xylose fermenting cells (Lee *et al.*, 2000; Ginovart *et al.*, 2011). In addition, *Saccharomyses cerevisiae* cells exhibit high alcohol concentration tolerance whereas the growth of *Pichia stipitis* cells is restricted at ethanol levels beyond 33 g/L (Walker, 1998; Lee *et al.*, 2000; Jeffries *et al.,* 2009; Ginovart *et al.*, 2011).

3.5.1 Batch-1 mashes fermentation characteristics

S. cerevisiae yeast fermentation kinetics for batch-1 worts monitored by CO_2 formation rates is presented in Fig. 3.11. For the KSV8 and SSV2 worts (control

samples), *S. cerevisiae* cells showed negligible yeast lag phase possibly because of the low sugar concentrations in the worts; the low sugar concentration tend to exerts minimal or negligible osmotic pressure on the cells (Ginovart *et al.,* 2011). SSV2 wort (control sample) showed higher and faster fermentation performance than the corresponding control KSV8 wort. However, CO_2 gas production rates reached a maximum within 12 h of fermentation commencement and started to decline afterwards (except for KSV8 substrate). The SSV2 wort with higher initial fermentable sugar than KSV8 has higher corresponding CO_2 gas and ethanol yields and no residual sugars were detected in the fermentation broth (Table 3.9). With regard to batch-1 fermentations, marginal yeast lag phase were observed for SSV2, KSV8 and KSV3 worts, SSV2 wort showed a relatively higher yeast lag phase that may be attributed to its high lignin content (Fig. 3.11). Lignin materials tend to liberate yeast inhibitory compounds such as phenols during mashing, these inhibitory compounds slowdown yeasts adaptation rates in media prior to fermentation commencement (Ginovart *et al.,* 2011). CO_2 gas formation rates were observed to reach their maximum peak within 24 h of fermentation time for the batch-1 worts. KSV8 followed by SSV2 yielded higher CO_2 gas and corresponding ethanol; these results were consistent with corresponding initial fermentable sugar levels of the substrates. Furthermore, no residual sugars were detected in the fermentation broths, however, residual FAN was detected which suggested that FAN was not the limiting nutrient in these fermentations (Table 3.9). Because *S. cerevisiae* cells were not xylose fermenting yeast, the xylose sugar concentration after fermentation appeared relatively unchanged.

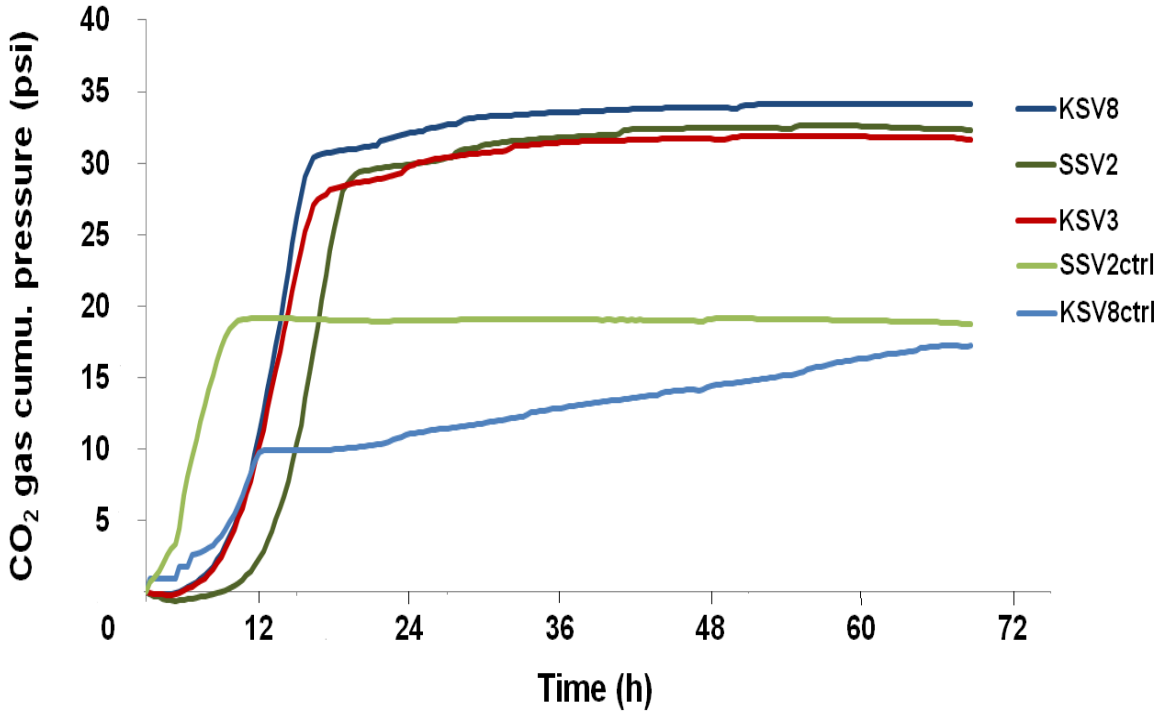


Fig. 3.11 Batch-1 sorghum mash fermentation kinetics. Fermentation kinetics of KSV8, SSV2 and KSV3 sorghum husked grains mashed with endogenous enzymes (control samples) and exogenous enzyme cocktails. Fermentation was by *S. cerevisiae* and monitored by ANKOM^{RF} (ANKOM Technology, USA) via measurement of CO_2 gas pressure.

Table 3.9 Batch-1 mashes fermentation yields by *S. cerevisiae* yeast

Parameter	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
	Batch-1: exogenous enzymes* **			**control: endogenous enzymes****	
Ethanol yield (L/t)	115.81^{a} ±2.37	120.46^{b} ±3.1	105.13^{c} ±2.7	53.27^{d} ±1.3	37.44^{e} ±1.51
CO_2 gas (kL/t)	26.01^{a} ±2.22	29.36^{a} ±3.10	24.40^{b} ±2.76	19.05^{c} ±1.6	11.70^{d} ±1.17
Glucose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Maltose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Xylose (g/100g flour)	3.93^{a} ±0.47	3.82^{a} ±0.79	4.14^{b} ±0.48	2.06^{c} ±0.65	2.65^{c} ±0.83
FAN (mg/L)	19.03^{a} ±1.77	17.57^{a}±2.0	22.18^{b}±1.89	ND	ND

***Exogenous enzymes mash comprised of commercially available enzyme cocktails. **endogenous enzymes are latent originally present in flour samples. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-e) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected

3.5.2 Batch-2 mashes fermentation characteristics

The increased sugars and FAN concentrations of batch-2 worts appeared to further enrich the substrates total nutrient values (Ijasan *et al.,* 2011). Consequently, fermentation lag phases were reduced to negligible and insignificant level for the SSV2, KSV8 and KSV3 worts (Fig. 3.12). Production rates of CO_2 gas appeared to reach maximum level within 24 h of fermentation time. KSV8 show higher and faster fermentation rate followed by SSV2. In terms of ethanol yields, the KSV8 substrates with relatively higher CO_2 gas production rate shows corresponding higher ethanol yield followed by SSV2 substrates (Table 3.10). The fermentation process appeared efficient because all the available glucose and maltose sugars were observed to be completely utilised i.e. no residual glucose and maltose sugars were detected in the final fermented broths. However, residual FAN was detected in the final broths; suggesting fermentable sugars are the limiting nutrients in the fermentation substrates (Table 3.10).

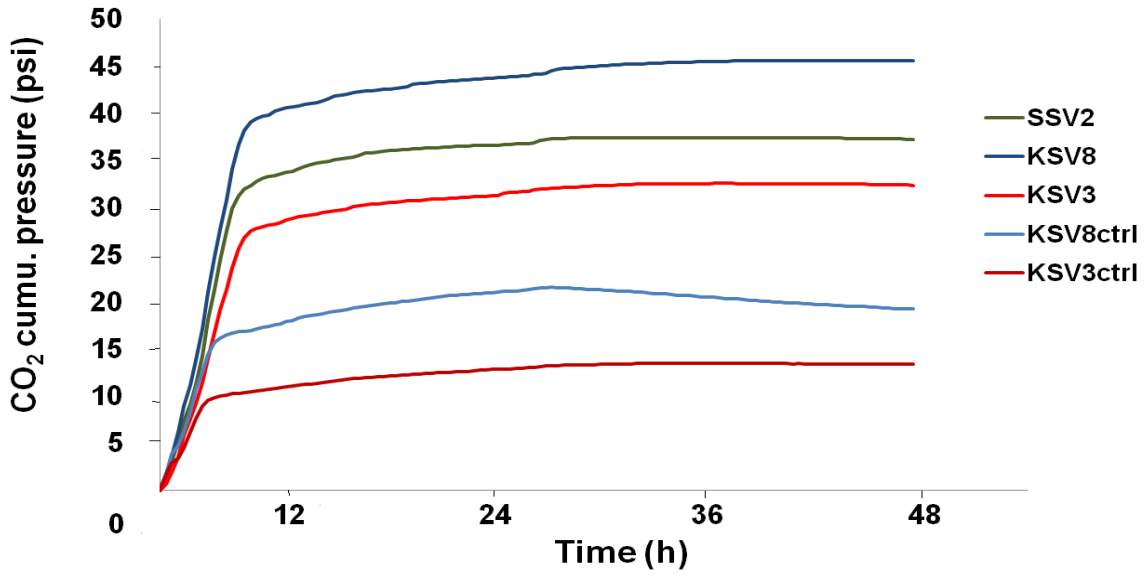


Fig. 3.12 Batch-2 sorghum mash fermentation kinetics. Fermentation kinetics of KSV8, SSV2 and KSV3 sorghum husked grains mashed with endogenous enzymes (control samples) and exogenous enzyme cocktails. Fermentation was by *S. cerevisiae* and monitored by ANKOM^{RF} (ANKOM Technology, USA) via measurement of CO_2 gas pressure formation.

Table 3.10 Batch-2 mashes fermentation yields by *S. cerevisiae* yeast

Parameter	SSV2	KSV8	KSV3	KSV8	KSV3
	Batch-2: exogenous enzymes***			Control: endogenous enzymes**	
Ethanol yield (L/t)	147.08^{a} ±2.41	151.32^{b} ± 2.82	126.43^{c} ±2.0	50.65^{d} ±2.14	40.71^{e} ±2.2
CO_2 gas (kL/t)	32.86^{a} ±2.81	38.80^{b} ±3.11	27.73^{c} ±3.44	18.30^{d} ±2.32	11.40^{e} ±2.7
Glucose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Maltose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Xylose (g/100g flour)	2.41^{a} ±0.52	2.77^{a} ±0.69	4.36^{b} ±0.78	2.58^{a} ±0.74	4.24^{b} ±0.89
FAN (mg/L)	41.89^{a} ±1.68	48.23^{b} ±2.11	52.45^{c} ±2.13	*ND	*ND

*******Exogenous enzymes comprised commercially available enzyme cocktails. While ******endogenous enzymes are latent originally present in flour samples. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected.

3.5.3 Batch-3 mashes fermentation characteristics

With the observed increase in substrate's FAN to about 150 mg/L level; the minimum required FAN level for efficient fermentation activity by yeasts (Thomas and Ingledew, 1992), *S. cerevisiae* and *P. stipitis* yeasts showed similar fermentation kinetics. This was in spite of previously reported slow adaptation of *P. stipitis* cells in fermentation media relative to *S. cerevisiae* (Agbogbo *et al*., 2006; Ginovart *et al.*, 2011). Perhaps, this may be because the worts sugar concentrations were not high enough to exert osmotic stress on the yeast cells (osmotic stress suppresses yeast efficient growth and metabolic activities). However, as the fermentation progresses to later phase, *S. cerevisiae* appeared to out-perform *P. stipitis* with regards to CO_2 formation rate from KSV8 substrate (Fig. 3.13). Perhaps this was due to *P. stipitis* sensitivity to higher ethanol concentration, which may reduce the cells ability to efficiently utilise xylose in the the presence of glucose (Caspeta *et al*., 2012),

subsequently. Only the glucose sugars were utilised by the *P. stipitis* cells as well. The data presented in Table 3.11 indicated that *P. stipitis* utilised all available glucose, maltose and some of the available xylose sugars. However, its observed ethanol yields were lower than equivalent fermentations with *S. cerevisiae*. This tended to suggest *P. stipitis* cells re-assimilated some proportion of the ethanol it produces in the late fermentation phase (Skoog *et al.*, 1992; Caspeta *et al*., 2012). In addition to *P. Stipitis* cells tendency to re-assimilate parts of the ethanol it produces, the yeast cells have higher respiratory capacity than *S. cerevisiae* yeasts. Thus, the *P. stipitis* cell tended to utilise more nutrients in cell maintenance and growth than *S. Cerevisiae* cells. Therefore, the combination of these factors perhaps resulted in *P. stipitis* producing relatively higher CO_2 gas (in respiration) than observed ethanol yield as shown in Table 3.11 (Skoog *et al.*, 1992; Jeffries *et al.*, 2009).

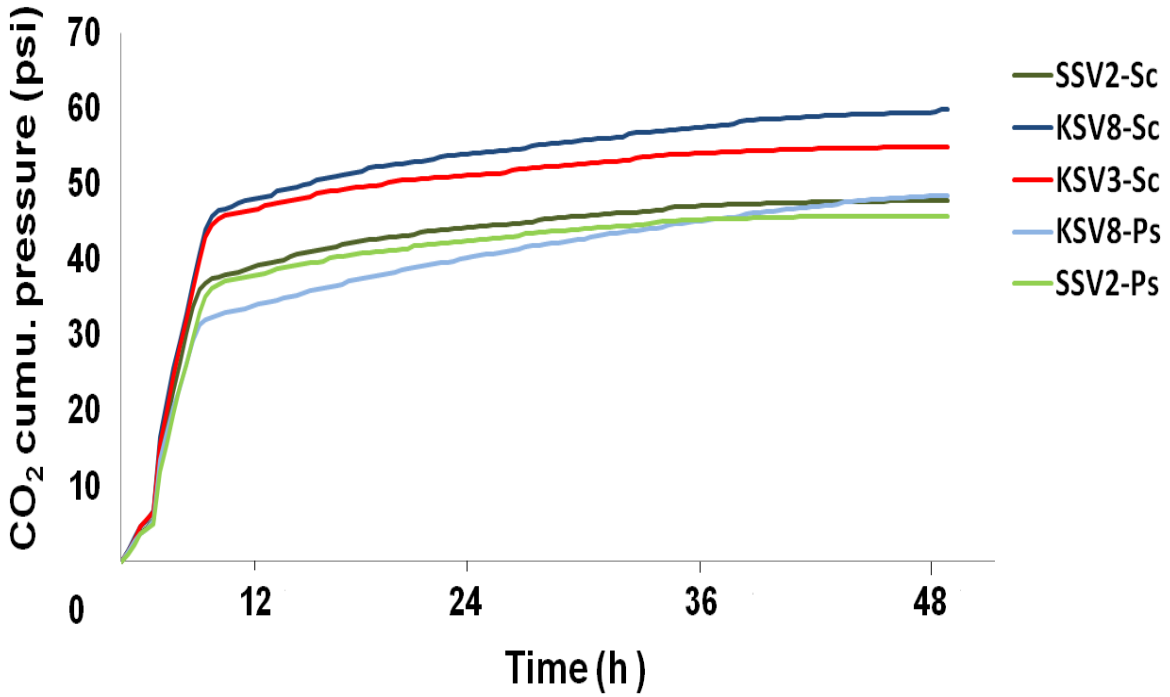


Fig. 3.13 Batch-3 sorghum mash fermentation kinetics. Fermentation kinetics of KSV8, SSV2 and KSV3 sorghum husked grains mashed with exogenous enzyme cocktails. Fermentation was by *S. cerevisiae* and *P. Stipitis* cells and monitored by ANKOM^{RF} (ANKOM Technology, USA) via measurement of CO_2 gas pressure formation rates.

Table 3.11 Batch-3 mashes fermentation yields.

Parameter	SSV2	KSV8	KSV3	SSV2	KSV8
	Fermented by *S. cerevisiae*			Fermented by *P. stipitis*	
Ethanol yield (L/t)	206.74^{a} ±2.0	260.12^{b} ±1.8	224.53^{c} ±1.7	185.89^{d} ±2.1	183.69^{d} ±1.2
CO_2 gas (kL/t)	41.45^{a} ±2.31	51.50^{b} ±3.56	46.81^{c} ±2.65	40.89^{d} ±3.14	39.77^{e} ±2.78
Glucose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Maltose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Xylose (g/100g flour)	3.06^{a} ±0.65	2.87^{a} ±0.43	4.68^{b} ±0.27	2.15^{c} ±0.46	3.26^{d} ±0.61
FAN (mg/L)	16.83^{a} ±0.98	13.36^{b} ±1.12	17.87^{a} ±2.0	17.24^{a} ±1.01	19.53^{c} ±2.06

Sorghum grain flours mashed with commercially available enzymes cocktail were fermented by *S. cerevisiae* and *P. stipitis* cells respectively. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-f) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test. *ND = Not Detected.

With regard to ethanol production, Fig. 3.14 show that *S. cerevisiae* cells performed better than *P. stipitis* cells in terms of KSV8 and SSV2 wort substrates. KSV8 substrates show the most favourable observed ethanol yield of about 31 g/L by *S. cerevisiae* yeast which was followed by KSV3 wort with 27 g/L and subsequently SSV2 wort with about 25 g/L respectively. However, *P. stipitis* showed maximum observed alcohol yield of about 22 g/L for both SSV2 and KSV8 worts. The observed relatively low fermentation performance of *P. stipitis* compared to *S. cerevisiae* appeared to be due to its tendency of reduced growth metabolism and fermentative capacity as ethanol concentration levels approached 30 g/L. Beyond alcohol concentration level of 30 g/L, *P. stipitis* cells tended to loose its fermentative capacity to efficiently utilised available xylose sugars because of reduced metabolic activities associated with high alcohol concentration stress, this is in spite of the observed residual FAN in the fermentation broths (Lee *et al*., 2000; Rouhollah *et al*., 2007).

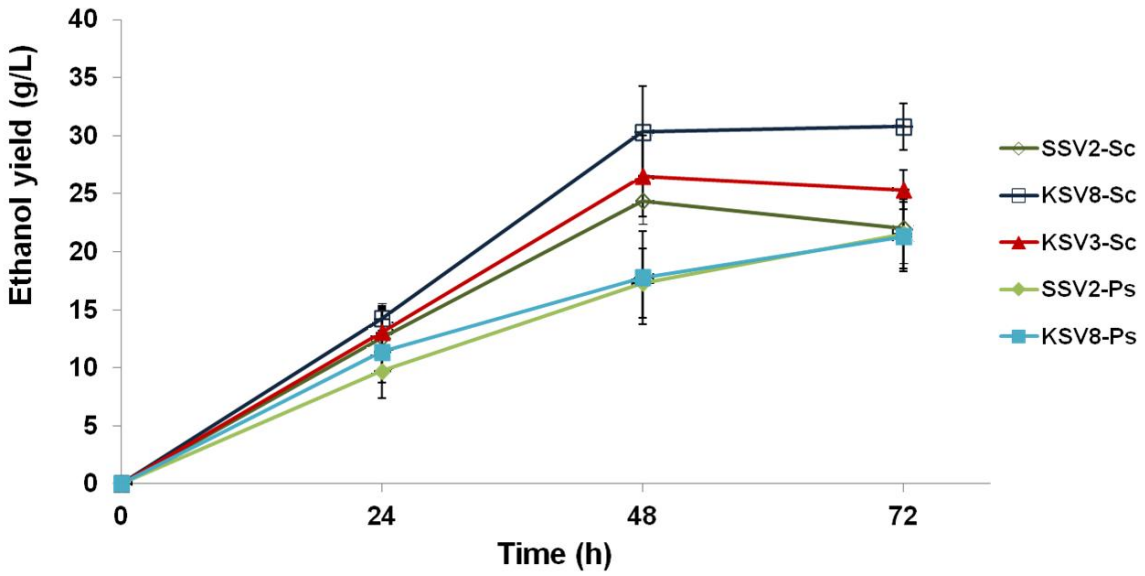


Fig. 3.14 Batch-3 sorghum mash fermentation profile. Fermentation profiles of KSV8, SSV2 and KSV3 sorghum husked grains mashed with exogenous enzymes cocktail. Fermentation was by *S. cerevisiae* (Sc) and *P. stipitis* (Ps) yeasts. Samples were withdrawn every 24 h for alcohol concentration determination by FermentoFlash® equipment.

3.5.4 Batch-4 mashes fermentation characteristics

As FAN level increased beyond 150 mg/L in fermentation worts (Table 3.8), fermentation rates of *S. cerevisiae* improved notably and yeast lag phase appeared negligible. However, *P. stipitis* cells showed relatively some notable lag phase (Fig. 3.15). Once again, this reflects the poor stress tolerance of *P. stipitis* compared to *S. cerevisiae* cells, which as a result of the former lower osmotic stress tolerance level compared to the latter (Caspeta, *et al*., 1992; Agbogbo *et al*., 2006). Batch-4 worts appeared to have higher glucose contents than batch-3 worts, thus, were likely to exert relatively higher osmotic stress to yeasts than the latter. Furthermore, the additional presence of lignin-derived phenolic compounds liberated during mashing (predominantly from husks materials) may further exacerbate environmental stress on the yeasts of which *P. stipitis* was reported to have less tolerance level for than *S. cerevisiae* (Hotz and Gibson, 2007; Ogbonna, 2011; Lyumugabe *et al.*, 2012). KSV8 wort showed higher CO_2 gas formation by *S. cerevisiae* fermentation followed by

KSV3 and SSV2 worts. Furthermore, the fermentation rates by *S. cerevisiae* for the three substrates were fast and relatively similar in terms of CO_2 production rates (Fig. 3.15). However, in spite of *P. stipitis* ability to partly utilise xylose sugar (Table 3.12), residual maltose was detected in its SSV2 and KSV8 fermentation broths; this is because *P. stipitis* yeasts ability to biosynthesize *maltase* enzymes that is required to split maltose into glucose molecules tended to diminished in the presence of high free glucose and alcohol concentrations in worts (Lee *et al*., 2000; Holtz and Gibson, 2007). Consequently, with increasing ethanol concentration level in fermentation media beyond the yeast tolerance threshold and in addition to the relatively high initial free glucose content in worts perhaps combined to suppress efficient fermentation of SSV2 and KSV8 by *P. stipitis* (Needleman, 1991).

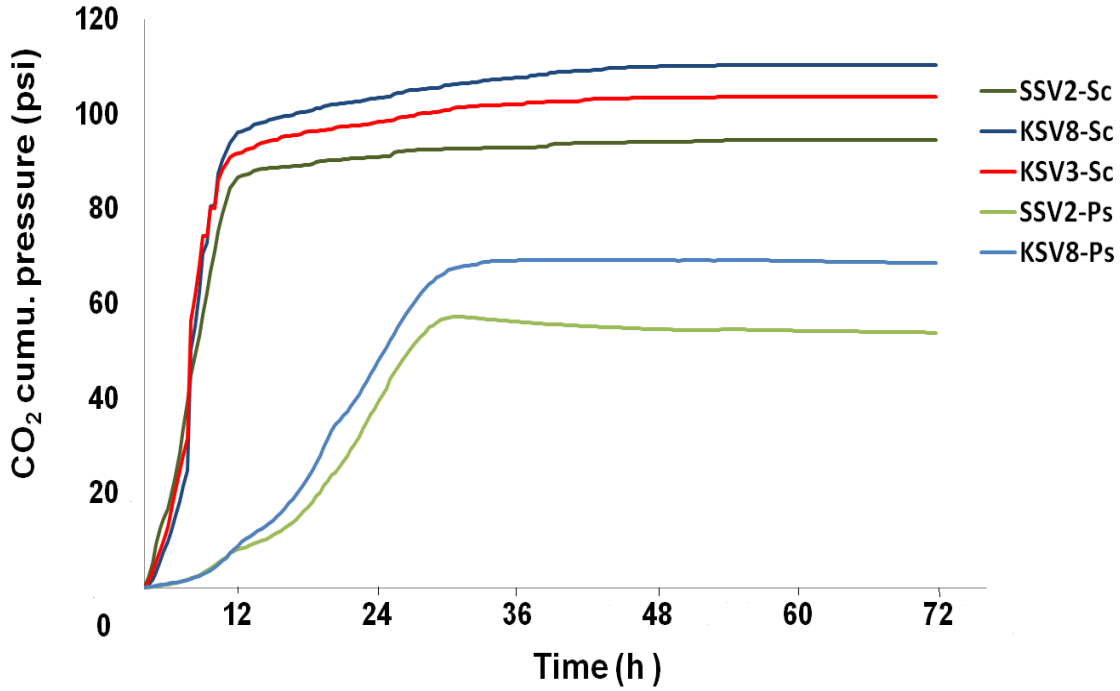


Fig. 3.15 Batch-4 sorghum mash fermentation kinetics. Fermentation kinetics of KSV8, SSV2 and KSV3 sorghum husked grains mashed with exogenous enzyme cocktails. Fermentation was by *S. cerevisiae* and *P. Stipitis* cells and monitored by ANKOM^{RF} (ANKOM Technology, USA) via measurement of CO_2 gas pressure formation rates.

Concerning ethanol yields, *P. stipitis* showed similar ethanol yield of about 32 g/L for SSV2 and KSV8 substrates (Fig. 3.16). These results corresponds to optimum ethanol yields of 30-35 g/L previously reported in literature for *P. stipitis* fermentation with starch based substrates (Lee *et al*., 2000; Rouhollah *et al.,* 2007; Gutiérrez-Rivera *et al.*, 2011). It is pertinent to reiterate that *P. stipitis* fermentative capacity tended to diminish as ethanol concentration reached 31 g/L and will usually appear to decline beyond that threshold (Skoog *et al.*, 1992; Rouhollah *et al.,* 2007; Caspeta *et al*., 2012). Therefore, it could be argued that high ethanol concentration would tend to be the limiting factor in *P. stipitis* fermentation rather than FAN or sugar availability in fermentation broths. Hence, residual maltose and xylose sugars as well as FAN were observed in SSV2 and KSV8 fermentation broths (Table 3.12).

However, SSV2, KSV8 and KSV3 substrates fermented by *S. cerevisiae* showed observed ethanol yields of about 42 g/L, 50 g/L and 45 g/L respectively (Fig. 3.16), these results corresponds to approximately 355 L/t, 421 L/t and 379 L/t respectively (Table 3.12). However, courtesy of Scotch Whisky Research Institute (SWRI, Edinburgh), SSV2, KSV8 and KSV3 samples were mashed with enzyme cocktails (not mentioned in Table 3.3) and fermented with selected industrial strain of *S. cerevisiae* in accordance to the SWRI standard methods. The SSV2, KSV8 and KSV3 showed improved ethanol yield of 425 L/t, 453 L/t and 434 L/t respectively. Furthermore, using 0.005M dilute sulphuric acid as the mashing liquor, the corresponding ethanol yields of 435 L/t, 465 L/t and 439L/t were obtained respectively (Courtesy SWRI, Edinburgh). Thus, the ethanol yields obtained by SWRI standard mashing and fermentation techniques were an improvement over the results initially reported in this study. The results suggested that there is room for improvement in the ethanol yield potentials of SSV2, KSV8 and KSV3 sorghum

substrates through the use of more efficient hydrolytic enzymes for mashing and robust fermenting yeasts. Furthermore, using mild sulphuric acid solution as mashing liquor resulted in improved ethanol yield because the acid solution will further hydrolyse grain's husks, awns, pubescence materials etc to liberate more fermentable sugars such as glucose and xylose in worts. Notwithstanding this, the reported ethanol yields of 355 L/t, 421 L/t and 379 L/t for SSV2, KSV8 and KSV3 sorghum samples in this study are an improvement over 360 L/t ethanol yield reported by Wu *et al.,* (2010), for de-husked and un-malted sorghum grain substrates supplemented with commercial enzymes and fermented by *S. cerevisiae*. However, Sheorain *et al.*, (2000) reported ethanol yields of 380-390 L/t for different de-husked and un-malted sorghum grain substrates which corresponds to this study reported results (i.e. 379 and 421 L/t for KSV3 and KSV8 samples). Other previous result findings reported ethanol yields of 460-490 L/t for malted and pearled sorghum grains that were mashed with exogenous enzyme supplements and fermented by industrial strain *S. cerevisiae* (Agu *et al*., 2006; Ogbonna, 2011; Okolo *et al*., 2011; Serna-Saldívar *et al*., 2011; Aregbesola *et al.*, 2012).

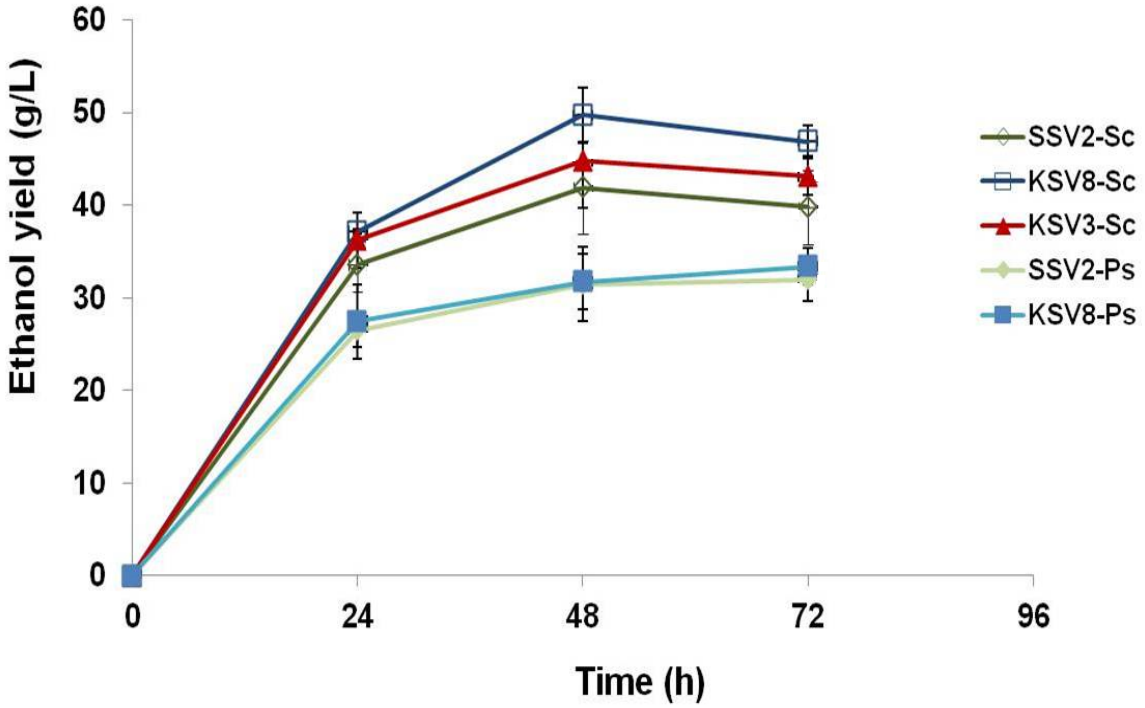


Fig. 3.16 Batch-4 sorghum mash fermentation profile. Fermentation profiles of KSV8, SSV2 and KSV3 sorghum husked grains mashed with exogenous enzymes cocktail. Fermentation was by *S. cerevisiae* (Sc) and *P. stipitis* (Ps) yeasts. Samples were withdrawn every 24 h for alcohol concentrations determination by FermentoFlash® equipment.

Table 3.12 Batch-4 mashes fermentation yields.

Parameter	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
	Fermented by *S. cerevisiae*			**Fermented by *P. stipitis***	
Ethanol yield (L/t)	354.67^{a} ±1.8	420.89^{b} ±2.9	378.49^{d} ±2.0	270.66^{c} ±1.9	272.11^{c} ±2.3
CO_2 gas (kL/t)	80.70^{a} ±2.34	93.90^{b} ±1.72	88.40^{c} ±2.34	48.60^{d} ±2.45	58.90^{e} ±1.98
Glucose (g/100g flour)	*ND	*ND	*ND	*ND	*ND
Maltose (g/100g flour)	*ND	*ND	*ND	6.85 ±1.16	7.94 ±1.02
Xylose (g/100g flour)	2.74^{a} ±0.71	2.81^{a} ±0.53	4.59^{b} ±0.86	2.18^{a} ±0.51	3.11^{c} ±0.69
FAN (mg/L)	10.31^{a} ±0.6	8.01^{b} ±1.0	10.43^{a}±1.2	15.99^{c} ±2.6	21.47^{d} ±2.0

Sorghum grain flours mashed with commercially available enzymes cocktail were fermented by *S. cerevisiae* and *P. stipitis* cells respectively. Sugars are determined by HPLC and FAN by Megazymes® kits. Means on the same row that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected.

3.6.0 Conclusion and recommendations.

In this study, the reported ethanol yields of 355, 421 and 379 L/t for SSV2, KSV8 and KSV3 sorghum crude grains compares favourably with ethanol yields obtained from fermentation of de-husked sound sorghum grains previously reported in scientific literature. Therefore, these result findings suggested that spoilt or degraded sorghum grains could be utilised as a low-cost feedstock source for bioethanol production. Interestingly, the results suggested that residual or degraded grains sourced from fields or storage facilities could be utilised directly for bioethanol production without prior investment in pre-treatment process such as de-husking, threshing, steeping and malting. Exclusion of these processes prior to fermentation of the substrates would not only save costs, it will reduce energy consumption that might otherwise be needed to carry out such processes. Less energy consumption is beneficial in both costs and GHG emission reductions. Utilisation of degraded or spoilt grains may be considered a sustainable route for disposal of spoilt grains by farmers whereby the farmers will be happy knowing that they have some economic relief when their farmlands or storage facilities are vulnerable. This measure will go a long way to drastically curb incidence of feeding livestock with spoilt grains or even humans.

Finally, to achieve even greater fermentation performance of SSV2, KSV8 and KSV3 crude husked grains, further fermentation techniques such as high gravity fermentation, immobilised yeast fermentation, exogenous nitrogen supplementation prior to fermentation, supplementing sorghum worts with malted barley prior to mashing and selection of efficient and robust yeasts may significantly improve ethanol yields, though at relatively higher production costs and likely with higher environmental degradation consequences.

CHAPTER FOUR

Bioconversion potential of whole sorghum crop residue (Bagasse) to ethanol

4.0.0 Introduction.

Global attention is focused on expanding the existing energy supply mix through harnessing of renewable energy sources such as solar, wind and biomass among others (Kothari *et al*., 2012). Petroleum, which contributes over 60% of road transport fuel globally, is considered a significant source of GHG emissions and this is generating serious concerns about global warming (Defra, 2008; Kothari *et al.*, 2012). Bioethanol, a plant-based liquid biofuel may be used in automobiles as additive or substitute to petroleum as transport fuel (Defra, 2006). Plant biomass such as grains, sugary stalk juices and lignocellulosic materials are potential feedstock sources for bioethanol production (Pandey *et al.,* 2011). However, concerns on food security relating to use of grains for ethanol production favoured the use of lignocellulose biomass for bioethanol production (Mousdale, 2008).

Sorghum is a water use efficient cereal with high potentials for lignocellulose biomass yield; it can be grown in 2-3 crop cycle per annum (Almodares *et al.,* 2008). On harvest, sorghum produces 65-120 t/ha of fresh lignocellulosic biomass, 3-12 t/ha of grains and also produces sugary stalk juice depending on the cultivar type (Billa, *et. al.*, 1997; Holou and stevens, 2012). Typical lignocellulosic biomass residue from sorghum harvest (i.e. mostly field leftovers) comprised crushed stalks (after juice extraction), stover, crop heads and leaves respectively. Combinations of these green residues were referred to as bagasse in this chapter. Sorghum crop

green residues typically comprised 27-25% hemicellulose, 34-44% cellulose and 18-21% lignin (Thanapimmetha *et al.,* 2011; Dogaris *et al.,* 2012; Heredia-Olea *et al*, 2013). Sorghum crop leaves contains appreciable starch and proteins than the stalks (Khan *et al.,* 2001). The celluloses and hemicelluloses are made up of polysaccharide polymers intertwined by tough lignin fibre (Yoshida *et al.*, 2008) which acts as barrier to hydrolysis of the celluloses and hemicelluloses materials thereby limiting fermentable sugar yields during the hydrolysis process (Phuengjayaem and Teeradakorn, 2011).

Previous studies have widely investigated various methods by which lignin can be degraded to make cellulose and hemicelluse polymers easily accessible for efficient enzymatic saccharification to liberate fermentable sugars that may be utilised in bioethanol production. However, most of these methods involved the use of chemicals to degrade the lignin materials, through which fermentation inhibitory compounds are generated which in turn results in poor fermentation performance of the hydrolysate substrates (Thanapimmetha *et al.,* 2011; Zhang *et al*., 2011). These generated fermentation inhibitory compounds (Fig. 4.1) may be classified into three groups; the Group 1 compounds comprise phenolic compounds such as syringic acid, syringaldehyde and vanillic acid generated as direct by-products from lignin breakdown. Group 2 compounds are furan derivatives consisting of furfural and 5-HMF which are mostly sugar degradation by-products that are generated during chemical hydrolysis of lignocellulosic biomass necessary to breakdown lignin while Group 3 compounds include organic acids such as acetic, formic and levulinic acids predominantly formed by hydrolysis of acetyl side-group and linkages in hemicellulosic "backbone" (Harmsen *et al.,* 2010; Chandel *et al*., 2011; Liang *et al.*, 2012).

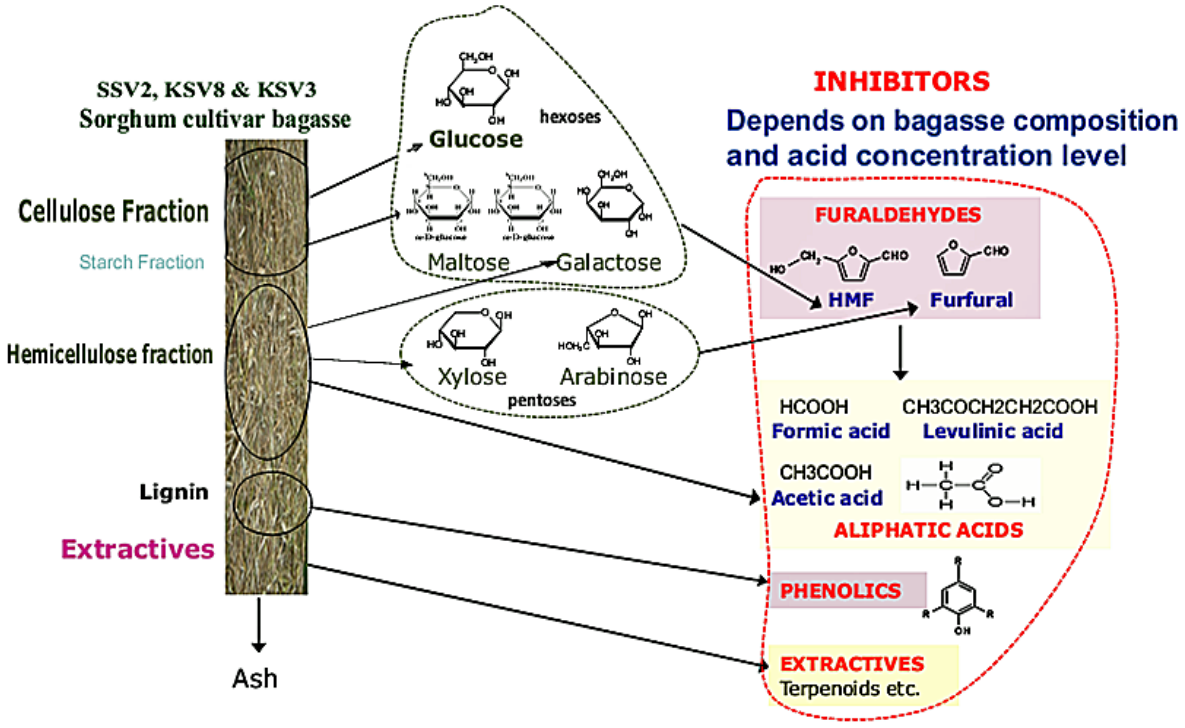


Fig. 4.1 Typical products of sorghum bagasse acid hydrolysis. Examples of fermentation inhibitory compounds generated during acid pre-treatment of lignocellulose biomass.

While pre-treatment methods such as catalyzed steam explosion, ammonia fibre explosion (AFEX), high energy radiation (e.g. ultrasound and microwave heating) among others are reported as effective methods of liberating fermentable sugars from lignocellulosic substrates with minimal formation of inhibitory compounds (Zheng *et al.,* 2009; Alvira *et al.,* 2010), the scale-up of these technologies to full commercial scales has been a serious challenge with regard to the overall process economy (Caspeta *et al*., 2013; Jung *et al*., 2013). However, conventional chemical pre-treatment method such as alkaline or acid hydrolysis of lignocellulose biomass is mostly considered economically feasible in terms of input chemicals and materials costs. However, the drawback in use of these technologies is their high potentials to generate fermentation inhibitory compounds during lignocellulose hydrolysis, of which the cost of the final hydrolysates detoxification process may outweigh the

overall economic benefits envisaged with these technologies (Harmsen *et al.,* 2010; Jung *et al.,* 2013). For example, the sulphuric acid pre-treatment method has been well studied and considered effective for lignocellulosic biomass hydrolysis (Panagiotopoulos *et al.*, 2010; Goshadrou *et al.,* 2011), but its downside includes generation of various inhibitory compounds such as phenols, furan derivatives and aliphatic acids during biomass hydrolysis (Zheng *et al*., 2009; Zacchi, 2011; Chandel *et al*., 2012). High or low (dilute) acid solutions may be employed for lignocellulosic biomass hydrolysis. However, whereas the high sulphuric acid concentration methods benefits from shorter substrates retention times (typically 10-45 min) and milder temperatures, the dilute sulphuric acid method has benefits of less inhibitory compounds generation and less corrosive acid solution to handle but required higher working temperatures (ranged between 160-220^{o}C) and longer substrates retention times, typically in the range of 4-10 h (Harmsen *et al.,* 2010; Zhang *et al.*, 2011; Wan *et al.,* 2012).

Consequently, the work describe in this chapter aimed to investigate the potential for utilisation of whole sorghum crop residue (bagasse) in bioethanol production through low-cost pre-treatment method. Therefore, dilute sulphuric acid pre-treatment method at relatively optimised low hydrolysis temperature followed by a low cost detoxification method was adopted in this study. The impact of bagasse cultivar type and varied crop cultivation location on bagasse fermentation performance were also investigated. The outcome of this study is expected to contribute towards the continued search for cost-effective and commercially viable lignocellulosic biomass pre-treatment methods and subsequently lead to improved fermentation performance of lignocellulose biomass.

4.1.0 Study background

Nigeria, the 2nd largest sorghum producer in the world generates millions of tons of sorghum residues after every harvesting season (Galadima *et al.,* 2011). According to estimates, 2-3 million metric tons of dry sorghum residues were generated annually in Nigeria. However, less than 30% of these were utilised as livestock feed and domestic fuel source for cooking, while the bulk of the remains were either left in the fields or burnt (Hyman, 1994; Yevich and Logan, 2003; Makinde *et al.*, 2011). The burning of agricultural residues in fields by farmers is largely considered a quick and labour-saving means of disposing of agricultural wastes. However, this act constitutes an environmental nuisance that usually results in poor air quality and by extension, impacts negatively on human health such as by causing breathing problems and poor visibility. Also, GHG emissions (considered a precursor to global warming) are also adding to the degree of concerns for field burning of agricultural wastes (Jenkins *et al.*, 1992; Kuhe *et al.*, 2013).

Therefore, this study envisaged that whole sorghum crop residues could be considered as a relatively cheap feedstock source for bioethanol production in Nigeria. Among benefits to be derived include expanded sorghum supply value chain in Nigeria i.e. in addition to the economic gains derivable by farmers through use of grains and stalk juices, the green wastes would now be of economic importance to the farmers as well. Furthermore, utilisation of sorghum residues as feedstock source for bioethanol production in Nigeria would contributes towards reduced environmental pollution associated with field burning of agricultural wastes which may lead to cost reduction in bioethanol production as well as addition to job creation opportunities via the emerging Nigerian bioethanol sector. According to NNPC report (2007), over 85% of bioethanol consumed in Nigeria (for gasoline blending) is

imported and this trend is not in tune with the country's ambitious target of achieving self-sufficiency in E-10 fuel blending by year 2020 (Nasidi *et al*., 2010; Ishola *et al.,* 2013).

Previous studies extensively investigated fermentation performance of Nigerian sorghum grains in brewing (Agu *et al.,* 2006; Ogbonna, 2011; Okolo *et al.,* 2011). However, very limited or no attention has been given to investigating the potential utilisation of whole sorghum residue e.g. crushed stalks, leaves, panicles and stover (bagasse) in bioethanol production (Adeosun, 2008; Aigbodion *et al*., 2010; Davila-Gomez *et al.,* 2011; Sathesh-Prabu and Murugesan, 2011; Kim *et al.,* 2012; Adeteju *et al.,* 2012). Furthermore, effect of crop cultivation location and cultivar type on fermentation performance of sorghum bagasse substrates has not been extensively investigated. Consequently, this PhD research study aimed to further investigate bioconversion potential of SSV2, KSV8 and KSV3 whole sorghum crops residues comprising leaves, crushed stalks, stover and panicles to ethanol.

4.2.0 Materials and Methods

4.2.1 Sorghum crop cultivation and harvest

SSV2, KSV8 and KSV3 sorghum cultivars were cultivated in Nigeria at Kano (site B) and Kaduna (site Z) under rain-fed conditions and with only cow dung application as fertilizer. Crop's physical parameters such as height, girth diameter, total green residue mass as well as monitoring of sites average diurnal temperatures and rainfall were done courtesy National Horticultural Research Institute (NIHORT, Nigeria). For maximum extractible stalk juice yields, crops were harvested before grains reached physiological maturation (i.e. when grains were at soft-dough stage). Consequently, SSV2 sorghum grains reached soft-dough stage 11 weeks after planting date while

KSV3 and KSV8 sorghum crops reached soft-dough stage at 16 weeks after planting date. Hence, the sorghum crops were harvested 11 and 16 weeks after planting dates accordingly. The fresh bagasse comprising crushed stalks, leaves, stover, peduncle and panicles were sun-dried for 2 days followed by oven drying at 60^{o}C for 48 h. The dried samples were hammer milled and finally sieved through 4 mm screen (Retsch, Germany). The bagasse samples moisture contents and total lignin was determined according to National Renewable Energy Laboratory standard analytical procedure (Hames *et. al.,* 2008). The crude proteins were determined by adding 2 g bagasse (dry wt.) into conical flasks containing 2M NaOH solution (50 mL), the mixtures were stirred at room temperature for 2 min followed by incubation in a rotary shaker at 120 rpm and 60^{o}C for 2 h. The final mixtures were centrifuged at 3800 rpm for 10 min, the supernatant (containing solubilised crude proteins) were collected and the protein concentrations determination by Bradford™ reagent (Sigma-Aldrich, UK) according to manufacturer's standard protocol. The total starch content was determined by Megazyme™ K-TSTA total starch assay in accordance to manufacturer's standard procedure (Megazymes®, Northern Ireland). Finally, bagasse pasting property was determined courtesy Scotch Whisky Research Institute Edinburgh (SWRI) by Newport scientific standard method (ST-00) using RVA-4™ Rapid Visco Analyzer equipment (Newport Scientific, Australia). An example of the methodology for KSV8 bagasse is:

1- Pre-dermined moisture content (MC) of bagasse = 11.23%.

2- Therefore, dry matter (DM) content = (100 - MC) = 88.77%.

3- Standard equation: required sample weight (S) = (3.0 × 86.0)/DM, where 3.0 and 86.0 are constants (SWRI, Edinburgh). Hence, S = (3.0 × 86.0)/90.14 = 2.91 g flour.

4- Weight of mixing water required W = (28.0 - S) = (28.0 - 2.91) = 25.09 g dH_2O.

Briefly, 2.91 g of bagasse was added into canister containing 25.09 g water. The suspension was homogenised by properly stirring with glass rod at room temperature. A paddle was placed into the canister and afterwards inserted into the Rapid Visco-Analyser for analysis. The instrument was switched on and allowed to pre-heat to 50^{o}C. Total analysis cycle time is 15 min. Refer to Table 3.1 (pp. 74) for the RVA cycle profile.

4.2.2 Bagasse pre-treatment and saccharification

Bagasse (20 g dry wt.) was added into glass conical flasks containing 2%v/v dilute H_2SO_4 acid (80 mL), the mixtures were incubated at 75^{o}C for 2 h with 150 rpm orbital shaking followed by addition of distilled water (30 mL) to the slurries and afterwards autoclaved at 121^{o}C for 15 min. Samples were withdrawn for sugar and FAN analysis and the acidic hydrolysates adjusted to pH 5.5 with anhydrous sodium hydroxide crystals. Enzyme cocktail (Table 4.1) was added into hydrolysates and the final volumes adjusted with distilled water to 200 mL, the resultant hydrolysates were incubated at 150 rpm orbital shaking for 20 h at 50^{o}C and the temperature adjusted to 60^{o}C to further incubate the slurry for 1 h

Table 4.1 Composition of hydrolytic enzymes

Enzyme	**activity**	**Dosage**	**Source**
Cellic® Ctec	(120 FPU/mL)[a]	1200 µL	Novozymes, Denmark
Cellic® Htec	(1090 FXU/mL)[b]	200 µL	Novozymes, Denmark
Promalt™ 295	(500 BGµ/mL-min)[c]	30 µL	Kerrys Biosciences, Ireland
Promalt™ 4TR	(300 BG µ/mL)	20 µL	Kerrys Biosciences, Ireland

[a]Filter paper unit.
[b]Fungal xylanan unit
[c]betaglucanase unit/mL

4.2.3 Bagasse hydrolysates detoxification

The enzymatic hydrolysates were over-limed to pH 10.0 with anhydrous $Ca(OH)_2$ followed by incubation with orbital shaking at 120 rpm for 15 min at 50^{o}C (Ge *et al.,* 2011), the final hydrolysates were adjusted to pH 6.0 with concentrated sulphuric

acid and the solution centrifuged at 3800 rpm for 10 min, the supernatants (100 mL) were transferred into conical flasks and activated charcoal (2.5 g) was added into the flasks and the mixtures swirled at room temperature for 3 min followed by incubation with orbital shaking at 150 rpm for 30 min at 50^{o}C. After further centrifugation at 3800 rpm for 10 min, the supernatant (hydrolysates) were filtered through vacuum pump equipped with GF/B Whatman glass microfiber filters. Samples (2 mL) were withdrawn from the filtrate for sugars, amino acids and FAN determination.

4.2.4 Sugars, free amino nitrogen (FAN) and amino acids determination

Free amino nitrogen (FAN) was determined by K-Large 02/11™ (yeast available nitrogen, YAN) and K-PANOPA 02/11™ (primary amino acid nitrogen, PAN) assay kits (Megazymes, Northern Ireland) according to the manufacturer's standard protocols. The total amino acids concentrations were determined courtesy of Heriot-Watt University Edinburgh. For glucose, xylose and arabinose sugars determination by HPLC, 1.0 mL hydrolysate (at 1:10 dilution) were filtered through 0.22 µm micro syringe filters into 2.0 mL vials containing 1 mL *meso*-erythritol solution (internal standard sugar). The final solutions were vortexed and placed in an HPLC auto sampler (Spectra-physics, USA) and the sugars separated with a 300 mm × 7.8 mm REZEX RPM-monosaccharide Pb+2 (8%) column^{TM} (Phenomenex, USA) and quantified using HPLC software (CSW32 version v.1.4 chromatogram software from DataApex®, USA).

4.2.5 Yeast seed culture preparation

Yeast seed cultures were prepared by separately inoculating two loop fulls each of *Pachysolen tannophilus* NCYC614 and *Saccharomyces cerevisiae* DCLM (courtesy of Kerry Biosciences, Menstrie, Scotland) into 400 mL YEPD media comprising

2.5%(w/v) bacteriological peptone, 2.5%(w/v) urea, 1.0%(w/v) yeast extract, 3.0%(w/v) glucose and 1.0%(w/v) xylose respectively. The cultures were incubated at 32^{o}C with orbital shaking at 150 rpm for about 28 h. Afterward, the yeast pellets were washed by suspending in distilled water and vortexed, the water was decanted and the procedure repeated twice.

4.2.6 Hydrolysates fermentation

Fermentation progress were monitored in parallel, by monitoring cumulative CO_2 pressure formation rates via ANKOM^{TM} system (ANKOM^{RF} TECHNOLOGY, USA) and by shaking flasks (in orbital shaking incubator) whereby samples were withdrawn every 24 h for ethanol determination by FermentoFlash® (Funke-Gerber^{TM}, Berlin). For the ANKOM^{RF} system fermentation set-up, hydrolysates (100 mL) were added into 250 mL ANKOM^{RF} glass bottles followed by inoculation of *P. tannophilus* and *S. cerevisiae* yeasts (1.0×10^{7} cell/mL) respectively into separate hydrolysate samples. The ANKOM^{RF} bottles were placed in a rotary shaking incubator set at 130 rpm and 32^{o}C, the fermentation progress was monitored by automated measurement of cumulative CO_2 gas pressure formation after every 20 min via ANKOM^{RE} gas-production system (ANKOM Technology, USA). Fermentations were allowed to run undisturbed until CO_2 gas production rate were observed to start declining.

However, for the Shaking flask fermentation set-up, 100 mL hydrolysates were added into 250 mL Erlenmeyer conical flask followed by inoculation of *P. tannophilus* yeasts and *S. cerevisiae* yeasts (1.0×10^{7} cell/mL) respectively into hydrolysates. The flasks top were plugged with cotton wool and placed into a rotary orbital shaking incubator set at 130 rpm and 32^{o}C. Samples were withdrawn after every 24 h for

ethanol concentration determination by FermentoFlash® equipment (Funke-Gerber™, Berlin). Fermentations were ended after 72 h, see Fig. 4.2 for methodology schematic representation.

Typical example of ANKOM CO_2 gas volume determination (SSV2B sample):

- Cumulative CO_2 gas pressure (P) = 29.076 psi = 200.472 kPa.
- Corresponding temperature (T) = 32.0°C = 305.0°K.
- Glass bottle: Rated volume = 250 mL; Actual volume = 310 mL (ANKOM Tech).
- Fermentation wort Vol. = 100 mL.
- Head-space volume in glass bottle (V) = 310 mL - 100 mL = 210 mL (0.21 litres).
- Gas constant (R) = 8.314472 $L{\cdot}kPa{\cdot}^{O}K^{-1}{\cdot}mol^{-1}$
- Number of gas moles (n) = P (V/RT) ------- Van der Waals gas law equation.
  thus, n = 0.01660 mol.
- From Avogadro's Law; 1 mole gas occupy 22.4 L volume.
- Hence, cumulative CO_2 gas produced (mL) per 100mL of wort = 0.01660 mol × 22.4 L/mol × 1000mL/L = 371.84 mL/10 g dry flour or 37.184 kL/t dry bagasse.

4.2.7 Ethanol concentration determination

Ethanol concentrations were determined by adding 11.0 mL fermentation broth into 20.0 mL beaker, alcohol probe was dipped into beaker and about 10.0mL broth were sucked into equipment's measuring cells. Finally, the ethanol concentration levels in broth were automatically analysed by equipment and the results printed out via integrated printer of the fermentorflash® equipment (Funke-Gerber™, Berlin).

4.2.8 Statistical analytical method

Significant difference between means was tested by ANOVA using Turkey method by Minitab™ 16 statistical software (MINITAB©, USA). Means that do not share a superscript letter (a-f) within same rows are significantly different ($p \leq 0.05$) based on grouping information using Tukey method at 95% simultaneous confidence interval.

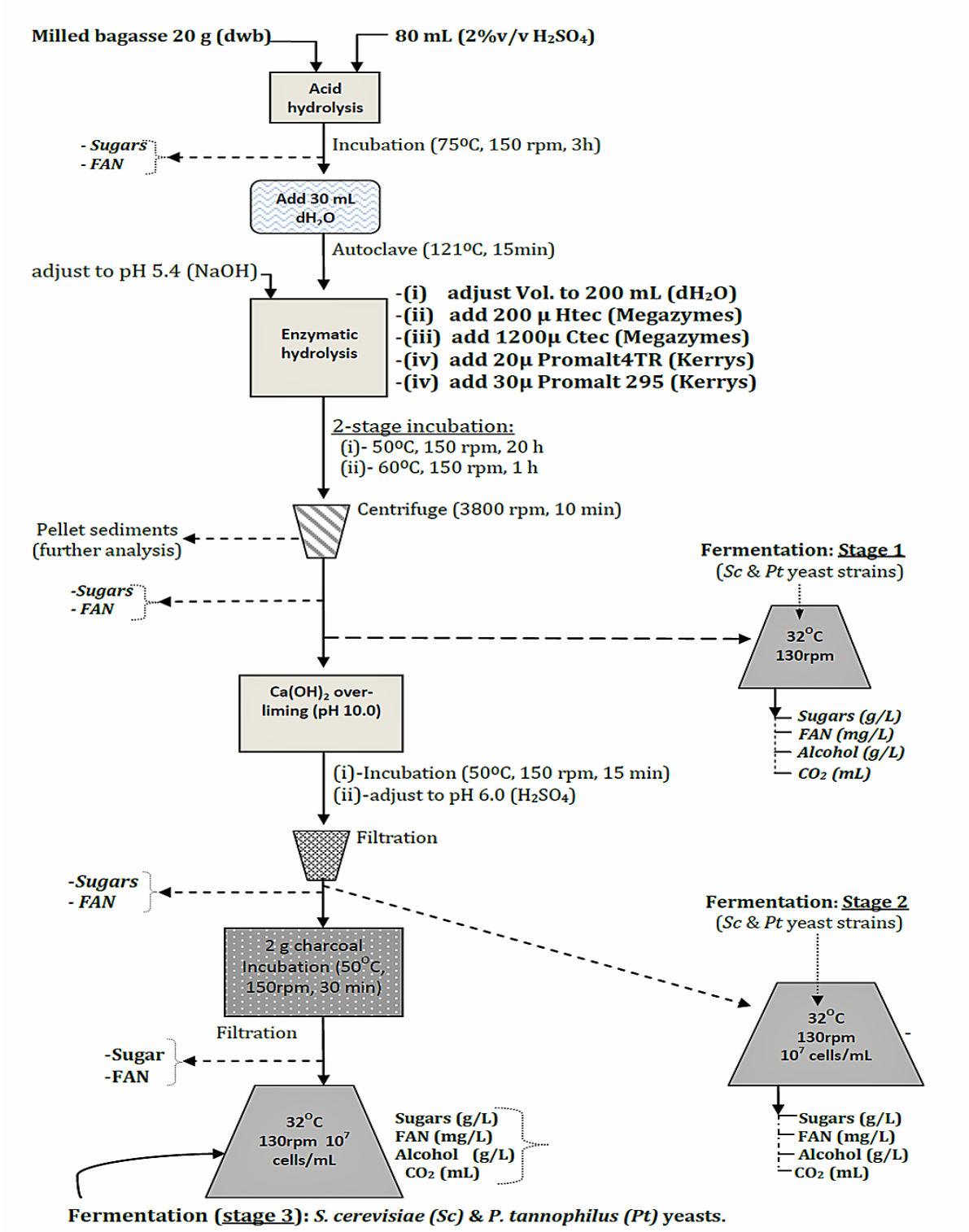


Fig. 4.2 An overview of sorghum bagasse pre-treatment processes. An overview of bagasse pre-treatment, saccharification and fermentation methodology employed in this thesis.

4.3.0 Results and discussions.

SSV2 sorghum grains reached soft-dough stage about 11 weeks after planting date while KSV8 and KSV3 sorghum grains were observed to be at their milk-stage. Consequently, allowing SSV2 sorghum to be harvested 16 weeks after planting (as may be the case for KSV8 and KSV3 sorghums) may results in grains being fully matured and substantial loss of the stalk juice being "dried up" (Almodares *et al*., 2010). This observation suggested SSV2 sorghum is a faster growing cultivar than KSV8 and KSV3. It may further be reiterated that stalks have maximum juice accumulation before their grains reach physiological maturation stage (Mazumdar *et al.,* 2012). The harvested soft-dough grains may be utilised to prepare local foods such as pap, tuwo etc (Olaoye and Oni, 2003). Data summarised in Table 4.2 indicated that SSV2 and KSV8 sorghums grow significantly ($p \leq 0.05$) taller and thicker in Kano than in Kaduna. These results are consistent with the bagasse samples starch contents. SSV2 and KSV8 sorghums have significantly ($p \leq 0.05$) higher starch contents in Kano than in Kaduna. These observations could be related to the sorghum agronomic characteristics. Sorghum, being a C4 crop tended to thrive better under drier and warmer climate that favours higher photosynthetic efficiency in C4 crops thereby leading to higher biomass formation (Almodares and Hadi, 2009; Ismaila *et al.,* 2010). Furthermore, results presented in Table 4.2 indicated that SSV2 and KSV8 bagasse have significanlty ($p \leq 0.05$) higher lignin content than in Kaduna. However, in spite of Kaduna soil being richer in nitrogen, potassium and available phoshorus content than Kano location soil (Table 2.1), KSV8 bagasse appeared to significantly ($p \leq 0.05$) contain higher protein content in Kano than in Kaduna. However, SSV2 bagasse contains higher protein content in Kaduna than Kano, a result that appeared consistent with the soil nitrogenous

contents of the two locations presented in Table 2.1. These results suggested that selection of the right sorghum cultivar along with suitable cultivation location such as choosing a drier and warm climatic condition may most likely favour higher sorghum bagasse and starch yields. However, the likely higher bagasse lignin content associated with warmer and drier climate as summarised in Table 4.2 may be a source of concern in terms of the ease of bagasse pre-treatment as fermentation substrate. Finally, soil quality (such as nitrogen content and porosity) also influnces nitrogenous content and growth characteristics of sorghum (Brink and Belay, 2006).

4.3.1 Bagasse pasting properties

There are very limited or no previous literature reports of sorghum bagasse pasting properties (Zeng *et al*., 2011). In spite of the low starch content of the bagasse samples (Table 4.2), celluloses are similar to amylose starch in terms of being made up of linear crystalline polysaccharide molecules, hence, cellulose and amylose polysaccharides tend to exhibit similar hydrolytic behaviours during mashing (Zaidul, *et. al.,* 2007; Winger *et al.,* 2009). However, KSV8B bagasse failed to completely paste, its failure to paste may possibly in part be associated with its high lignin content and very low starch content relative to the other substrates as summarised in Table 4.2 (Winger *et al.,* 2009; Hasjim *et al.*, 2013). However, the KSV8Z which has relatively lower lignin and higher starch content than KSV8B showed an "irregular" viscogram that is totally different from those of KSV3 and SSV2 bagasse (Fig. 4.3). Furthermore, SSV2 and KSV3 substrates show similar viscogram patterns (Fig. 4.4). The pasting temperatures and peak times of SSV2 and KSV3 bagasse corresponds to that of sorghum grains starch (Agu, *et. al.,* 2006; Zaidul, *et. al.,* 2007; Van Hung, 2008; Zeng, *et. al.,* 2011). However, KSV8Z showed a very short peak time results that is consistent with its very low starch content i.e.

the starch quantity is not sufficient to maintain longer pasting time (Table 4.3). The SSV2B and KSV3B show lower setback viscosities than SSV2Z (Table 4.3) possibly suggesting Both former substrates contained higher amylose and cellulose polymers than the latter, this is because amylose and cellulose are crystalline polysaccharide molecules that require longer time to re-aligned back to their crystalline structure during retrogradation or paste cooling phase i.e. after gelatinization (Pongsawatmanit, *et al.*, 2002; Santillán-Moreno *et al.*, 2011).

Table 4.2 Sorghum bagasse physico-chemical composition

	Kano			Kaduna	
Parameter	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Cultivation	11 weeks	16 weeks	16 weeks	11 weeks	16 weeks
Crop height (m)	$1.80^{a} \pm 0.05$	$3.20^{b} \pm 0.07$	$3.60^{c} \pm 0.04$	$1.62^{d} \pm 0.04$	$2.81^{e} \pm 0.04$
Diameter (cm)	$1.95^{a} \pm 0.10$	$2.62^{c} \pm 0.11$	$2.79^{c} \pm 0.03$	$1.80^{d} \pm 0.10$	$2.51^{e} \pm 0.02$
*Fresh bgs (t/ha)	$41.72^{a} \pm 3.1$	$48.31^{b} \pm 2.6$	$52.32^{c} \pm 1.1$	$37.06^{d} \pm 2.8$	$45.78^{e} \pm 1.4$
**Dry bgs (t/ha)	$28.60^{a} \pm 1.1$	$32.72^{b} \pm 0.8$	$36.83^{c} \pm 1.5$	$24.31^{d} \pm 0.9$	$30.49^{e} \pm 1.2$
Total starch: %	$5.14^{a} \pm 0.54$	$1.09^{b} \pm 0.06$	$3.16^{c} \pm 0.21$	$4.17^{d} \pm 0.14$	$0.78^{e} \pm 0.05$
Total lignin: %	$18.40^{a} \pm 0.3$	$21.65^{b} \pm 0.2$	$18.70^{a} \pm 0.6$	$16.86^{d} \pm 0.4$	$19.41^{e} \pm 0.3$
Total protein %	$4.61^{a} \pm 0.2$	$3.53^{b} \pm 0.16$	$3.24^{b} \pm 0.12$	$5.23^{c} \pm 0.16$	$2.69^{d} \pm 0.21$

Bagasse properties of SSV2, KSV8 and KSV3 sorghums cultivated in Kano and Kaduna under varied climate conditions. *Fresh bgs: fresh bagasse (leaves, crushed stalks, stover and panicle). **Dry bgs: oven dried bagasse. Results are Std. means of triplicate experiments. Means on the same row that do not share same superscript letter (a-e) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

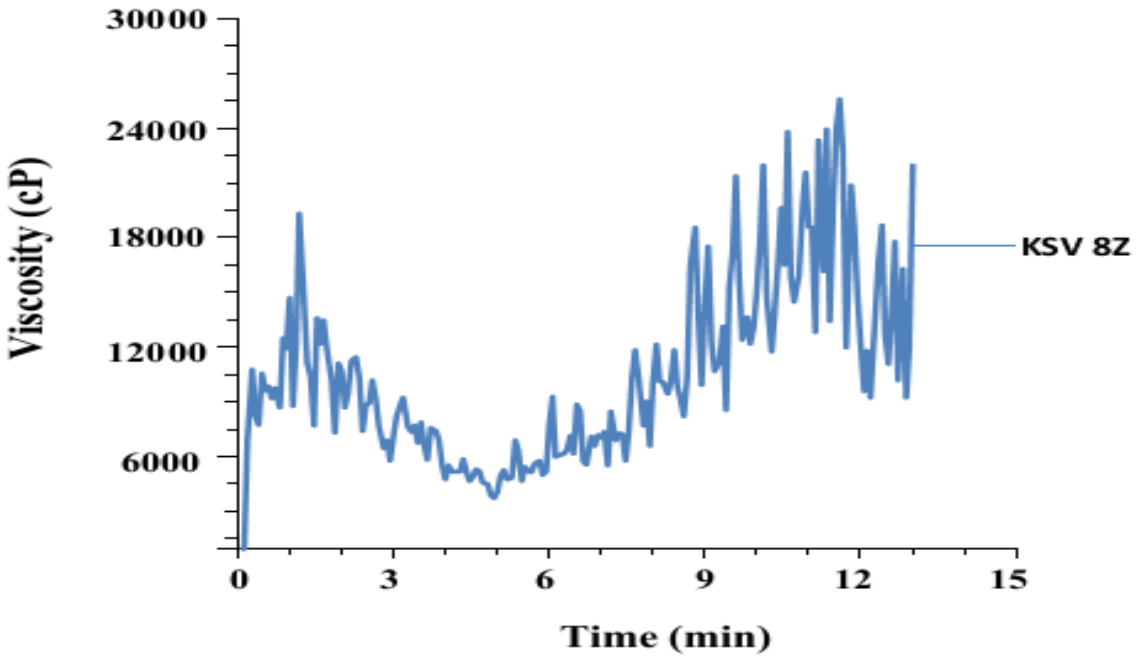


Fig. 4.3 KSV8 sorghum bagasse viscogram profile. Pasting profile of KSV8 sorghum bagasse cultivated in Kaduna (Nigeria), determined by rapid visco analyzer (model 3D+) in accordance to SWRI standard procedure. Table 3.1 provides the RVA temperature profile. Std. means of duplicate experiments.

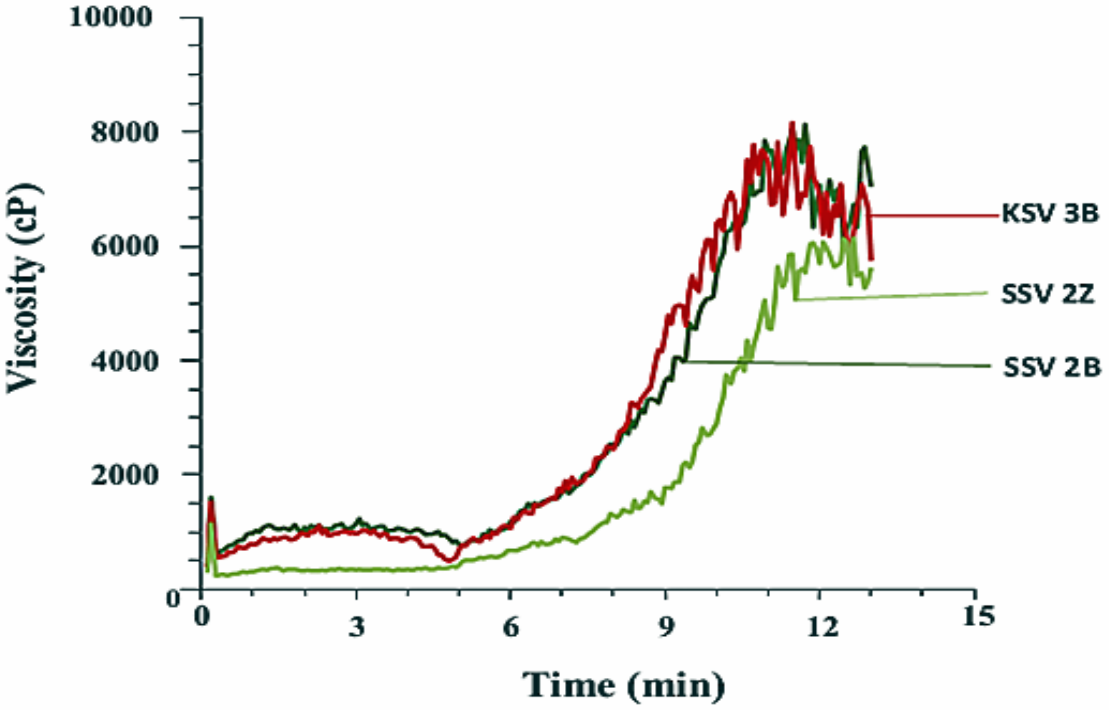


Fig. 4.4 SSV2 and KSV3 sorghum bagasse viscogram profile. Pasting profile of SSV2, KSV8 and KSV3 sorghum bagasse determined by rapid visco analyzer (model 3D+) in accordance to SWRI standard procedure. Table 3.1 provides the RVA temperature profile. Std. mean of duplicate experiments.

Table 4.3 Sorghum bagasse pasting viscosities

Crop	Peak viscosity (cP)	Set-back viscosity (cP)	Pasting Temp (^oC)	Peak time (min)	Final viscosity (cP)
SSV2-B	$1706^a \pm 11$	$5861^a \pm 19$	$50.45^a \pm 0.1$	$7.00^a \pm 0.4$	$7042^a \pm 14$
KSV3-B	$1771^b \pm 14$	$4541^b \pm 21$	$49.90^a \pm 0.2$	$6.93^a \pm 0.3$	$5756^b \pm 13$
SSV2-Z	$910^c \pm 13$	$4963^c \pm 17$	$50.35^a \pm 0.1$	$7.00^a \pm 0.5$	$5636^c \pm 12$
KSV8-Z	$19320^d \pm 22$	$16549^d \pm 20$	$49.95^a \pm 0.1$	$1.20^b \pm 0.1$	$22073^d \pm 17$

Means in the same column that do not share same superscript letter (a-d) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

4.3.2 Bagasse hydrolysis and detoxification

The choice of mild sulphuric acid pre-treatment in this study was principally based on cost considerations. Sulphuric acid is amongst the cheap and affordable chemicals that may be use for bagasse pre-treatment at cost-effective rates and relatively less inhibitory compounds generation during hydrolysis as compared to high acid concentration pre-treatment that tend to generates higher toxic compounds in hydrolysates (Ge *et al.,* 2011; Chandel *et al.,* 2011; Thanapimmetha *et al*., 2011). Dilute or mild acid pre-treatment methods are usually associated with high hydrolysis temperatures, typically above 160^{o}C. However, high hydrolysis temperatures results is higher formation rates of furan derivative compounds during hydrolysis. Another disadvantage of higher hydrolysis temperature is the possibility of denaturing the substrate's protein compounds which impacts negatively on the subsequent nutrient quality of the final hydrolysates (Chandel *et al.,* 2011; Cao *et al*., 2012). Phenolic compounds generated during lignin degradation by acid pre-treatment may be removed by detoxification process of the final hydrolysates. Several hydrolysates detoxification processes have been reported (Thanapimmetha *et al*., 2011; Ge *et al.,* 2011; Chandel *et al.,* 2011).

In this study, optimised low hydrolysis temperature (less than 100^{o}C) were chosen to minimise fermentation inhibitory compounds generation whilst maintaining bagasse

hydrolysis efficiency. Over-liming followed by charcoal filtration of hydrolysates was adopted as detoxification method in this study.

Acidic hydrolysates

Sorghum bagasse typically comprised 27-25% hemicellulose, 34-44% cellulose and 18-21% lignin (Thanapimmetha *et al.,* 2011; Dogaris *et al.,* 2012; Heredia-Olea *et al*, 2013). Acid hydrolysis pre-treatment reportedly (Harmsen *et al.,* 2010) liberates most of the pentose sugars (i.e. xylose and arabinose) and furan derivatives along with weak acids from bagasse hemicellulose materials. However, very little hexose sugars are liberated from the bagasse cellulose materials due to acid hydrolysis (Chandel *et al*., 2011). Furthermore, only limited amount of free amino nitrogen (FAN) are normally liberated from crude proteins by acid hydrolysis as protease enzymes would be required to degrade the crude protein polymers (Thomas and Ingledew, 1992; Mamma *et. al.,* 2009). Consequently, the sugar yields of 21-26 g/100g bagasse (Table 4.4) reported in this chapter corresponds to previously reported total sugar yields of 18-29 g/100g sorghum bagasse pre-digested with 2% (v/v) NaOH solution for 2 h and hydrolysed by 21.44% (v/v) H_2S0_2 solution for about 73 min (Thanapimmetha *et al.,* 2011). However, Ban *et al*., (2008) reported higher total sugar yields of 30 g/100g sorghum bagasse hydrolysed with 90% of 80 g/L phosphoric acid at 120^{o}C for 80 min. These results suggested improved sugar yields from acid pre-treatments may be achievable with SSV2, KSV8 and KSV3 sorghum bagasse such as by employing slight increases in of acid concentration or additional chemical use.

i. Enzymatic hydrolysates.

For improved glucose and maltose sugars liberation, the acidic hydrolysates were further hydrolysed with hydrolytic enzymes (Table 4.1). While cellulose polymers are hydrolysed by *cellulases,* the starch carbohydrates were hydrolysed by *amylases* while residual hemicelluloses polymers were hydrolysed by *hemicellulases* (Goshadrou *et al.*, 2011; Gao *et al.,* 2011). Additionally, proteolytic enzymes would degrade proteins polymers to simple molecules thereby increasing yeast available nitrogen levels in the hydrolysates (Goldammer, 2008). Thus, significant increase in the hexose sugars and FAN levels were observed after enzymatic hydrolysis (Table 4.4). The SSV2B substrates glucose yield (47 g/100g bagasse) and corresponding pentose sugar yield (23 g/100g bagasse) is relatively lower than previously reported glucose yield of 59 g/100g and pentose sugar yield of 27 g/100g bagasse reported by Phuengjayaem and Teeradakorn (2011) for sorghum bagasse pre-treated by ammonium explosion (AFEX) and hydrolysed with enzyme cocktails for 7 days; although their results obviously appeared more favourable than the reported values here (Table 4.6), our methods required less material resource input and is less time consuming relative to the previosuly reported method.

Furthermore, the observed glucose and pentose sugar yields (Table 4.4) compare favourably with those of Panagiotopoulos *et al.*, (2010), wherein they reported glucose yields of 31 g/100g and pentose sugar yields of 15 g/100g for sorghum bagasse pre-treated with 10% NaOH (w/w) solution for 24 h followed by enzymatic hydrolysis. Similar results were reported by Liang *et al.,* (2012); they reported glucose and xylose sugars yields of 32 g/100g and 13 g/100g sorghum bagasse respectively. The bagasse samples were steeped in solution comprising 0.1 g lime/g bagasse and 10 mL water/g bagasse for 2 h followed by 3 days enzymatic hydrolysis

with cocktail of enzymes. Several glucose yields ranging from 23-61 g/100g and pentose yields of 44-37g/100g sorghum bagasse pre-treated by various methods and hydrolysed by different enzymes under different chosen conditions were reported in the scientific literature and most of these results compared favourably with our findings presented in Table 4.4 for enzymatic hydrolysates (Sipos *et al*., 2009; McIntosh and Vancov, 2010; Zhang *et al.,* 2011; Saini *et al.,* 2013).

Table 4.4 Initial sugar content of SSV2, KSV8 and KSV3 hydrolysates (g/100g bagasse)

Bagasse	Hydrolysates	Glucose	Xylose	Arabinose	Total sugars
	Acidic	8.82^{a} ±1.1	13.46^{a} ±0.4	3.49^{a} ±0.6	25.77^{a} ±0.8
	Enzymatic	46.46^{ab} ±1.1	17.29^{ab} ±0.5	5.45^{b} ±0.5	69.19^{c} ±1.1
SSV2B	$Ca(OH)_2$ Overlimed	43.85^{af} ±1.0	15.06^{cd} ±0.9	5.27^{b} ±0.9	64.18^{ab} ±2.6
	Charcoal filtrate	42.88^{af} ±1.0	13.70^{a} ±0.2	5.08^{b} ±1.0	61.66^{bc} ±2.2
	Acidic	9.82^{a} ±1.0	12.35^{b} ±0.4	3.22^{a} ±0.2	25.39^{a} ±0.8
	Enzymatic	44.03^{ac} ±2.1	16.86^{ab} ±1.1	5.19^{b} ±0.1	66.07^{d} ±0.8
SSV2Z	$Ca(OH)_2$ Overlimed	42.07^{af} ±0.5	14.14^{c} ±1.5	4.96^{b} ±0.9	61.16^{bc} ±2.9
	Charcoal filtrate	41.76^{af} ±1.0	12.11^{e} ±0.2	4.03^{c} ±0.1	57.88^{cd} ±1.2
	Acidic	1.54^{b} ±0.2	15.35^{c} ±0.1	4.01^{c} ±0.6	20.89^{b} ±0.9
	Enzymatic	26.57^{ad} ±1.2	21.22^{ac} ±1.1	6.44^{d} ±0.4	54.22^{e} ±2.8
KSV8B	$Ca(OH)_2$ Overlimed	23.25^{cf} ±0.9	17.87^{ab} ±0.9	6.34^{d} ±0.1	47.46^{ad} ±1.8
	Charcoal filtrate	22.84^{cf} ±1.0	15.80^{c} ±1.2	5.76^{b} ±0.2	44.40^{fe} ±0.3
	Acidic	2.61^{c} ±0.7	14.54^{c} ±0.7	3.62^{a} ±0.2	20.75^{b} ±1.2
	Enzymatic	24.38^{bc} ±0.8	20.37^{ac} ±1.7	5.38^{b} ±0.3	50.14^{f} ±1.2
KSV8Z	$Ca(OH)_2$ Overlimed	22.13^{cf} ±0.9	16.91^{ab} ±0.4	5.33^{b} ±0.6	44.37^{fe} ±1.9
	Charcoal filtrate	21.80^{cf} ±0.1	14.09^{c} ±0.7	5.03^{b} ±0.2	40.91^{ce} ±0.9
	Acidic	8.36^{a} ±0.6	13.81^{a} ±0.7	3.47^{a} ±0.2	25.64^{a} ±0.9
	Enzymatic	44.62^{ac} ±0.8	16.94^{ab} ±1.1	5.23^{b} ±0.3	66.79^{d} ±1.2
KSV3B	$Ca(OH)_2$ Overlimed	42.08^{af} ±0.9	15.20^{c} ±0.2	5.06^{b} ±0.6	62.34^{bc} ±1.7
	Charcoal filtrate	42.03^{af} ±0.3	14.01^{c} ±0.6	4.87^{b} ±0.7	60.88^{bc} ±1.6

Milled and oven-dried sorghum bagasse pre-treated with dilute H_2SO_4 acid followed by enzymatic saccharification then overliming with $Ca(OH)_2$ and finally filtered with activated charcoal. Sugars were determined by HPLC. Corresponding Means in the same column that do not share same superscript letter (a-f) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

ii. Overlimed hydrolysates.

Organic acids and furan derivatives generated in acid hydrolysis are toxic to yeast cells and result in sluggish or stuck fermentation performance (Mehmood *et al.,* 2009; Davies *et al*., 2011), therefore, we employed over-liming technique to

precipitate out these acids as salts. This method is considered cost-effective but with resultant slight loss of fermentable sugars and nitrogenous compounds in the hydrolysates, sugars and proteins precipitates out along with the organic acids salts. Therefore, excessive over-liming of hydrolysates may not necessarily result in 100% removal of toxic compounds but may rather results in increased loss of sugars and nitrogenous compounds from the hydrolysates (Chandel *et al*., 2011; Ge *et al*., 2011). Consequently, despite our optimising the over-liming dosage rate in the preliminary study, a 5-7% decline in fermentable sugars and FAN levels in SSV2, KSV8 and KSV3 bagasse hydrolysates were indicated by data summarised in Tables 4.4-5. However, some literature findings reported negligible change in fermentable sugars level after over-liming, for example, Mehmood *et al*., (2009) reported initial glucose concentration of 46 g/L sorghum bagasse hydrolysate and 45 g/L after over-liming with $Ca(OH)_2$.

Table 4.5 Initial free amino nitrogen (FAN) of bagasse hydrolysates (mg/L)

	Kano			Kaduna	
Hydrolysates	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Acidic	130.3^{a} ±3.1	91.9^{b} ±1.9	123.2^{c} ±1.8	122.7^{c} ±2.5	83.4^{d} ±1.7
Enzymatic	251.8^{a} ±3.8	180.4^{b} ±2.1	248.0^{c} ±2.6	254.4^{a} ±3.2	163.5^{d} ±1.3
$Ca(OH)_2$ Overlimed	238.4^{a} ±3.6	168.0^{b} ±1.9	236.4^{a} ±2.8	240.5^{c} ±3.1	151.2^{e} ±2.0
Charcoal filtrate	205.8^{a} ±1.8	146.4^{b} ±2.1	188.0^{c} ±2.7	211.4^{d} ±2.2	139.5^{e} ±1.6

Milled oven-dried sorghum bagasse pre-treated with dilute H_2SO_4 acid followed by enzymatic saccharification and overlimimed with $Ca(OH)_2$ and finally filtered with activated charcoal. FAN were determined by K-PANOPA™/K-LARGE™ Megazymes® kits. Means on the same row that do not share same superscript letter (a-e) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test.

iii. Charcoal treated hydrolysates.

The phenolic compounds generated from degradation of lignin by acid hydrolysis of bagasse are toxic to efficient yeast metabolism (Chandel *et al.*, 2011), these compounds impart a deep brown colour to the hydrolysates as shown in Fig. 4.5 (Zhao *et al.,* 2011). For improved fermentation efficiency, phenols were removed by

charcoal filtration in this work. Charcoal filtration is a cost-effective phenols removing method from fermentation hydrolysates. However, like over-liming methods, its drawback include slight loss of sugars and FAN molecules during charcoal filtration, sugar and protein molecules gets adsorbed at the charcoal surface area and get removed from hydrolysate solution along with the charcoal particulates (Alvira *et al*., 2010; Ge *et al.,* 2011). Subsequently, a further loss of 7-10% of sugar and FAN concentrations was observed relative to over-limed hydrolysate compositions (Tables 4.4-5). Despite using equal charcoal treatment dosage rates and treatment conditions for the over-limed hydrolysates in this study, the final hydrolysates after charcoal filtration appeared with varied degree of colour intensities as shown in Fig. 4.6. This may be as a result of lignin being a very complex 3-dimensionally structured polyphenolic molecule whose specific chemical properties may vary from one plant tissue to another (Ellen, 1991; Mussatto *et al*., 2010). The observed colours in hydrolysates suggested presence of residual phenols in the solution, further phenols compounds may be removed through additional treatment of the hydrolysates with charcoal. However, this will lead to additional loss of sugars and FAN from hydrolysates which will certainly limit potential ethanol yield achievable from the hydrolysate substrates.

Fig. 4.5 Sorghum bagasse hydrolysate after over-liming treatment. SSV2, KSV8 and KSV3 sorghum bagasse cultivated in Kano and Kaduna (Nigeria), Nigeria, hydrolysed by mild acid and detoxified by overliming with anhydrous calcium hydroxide crystals.

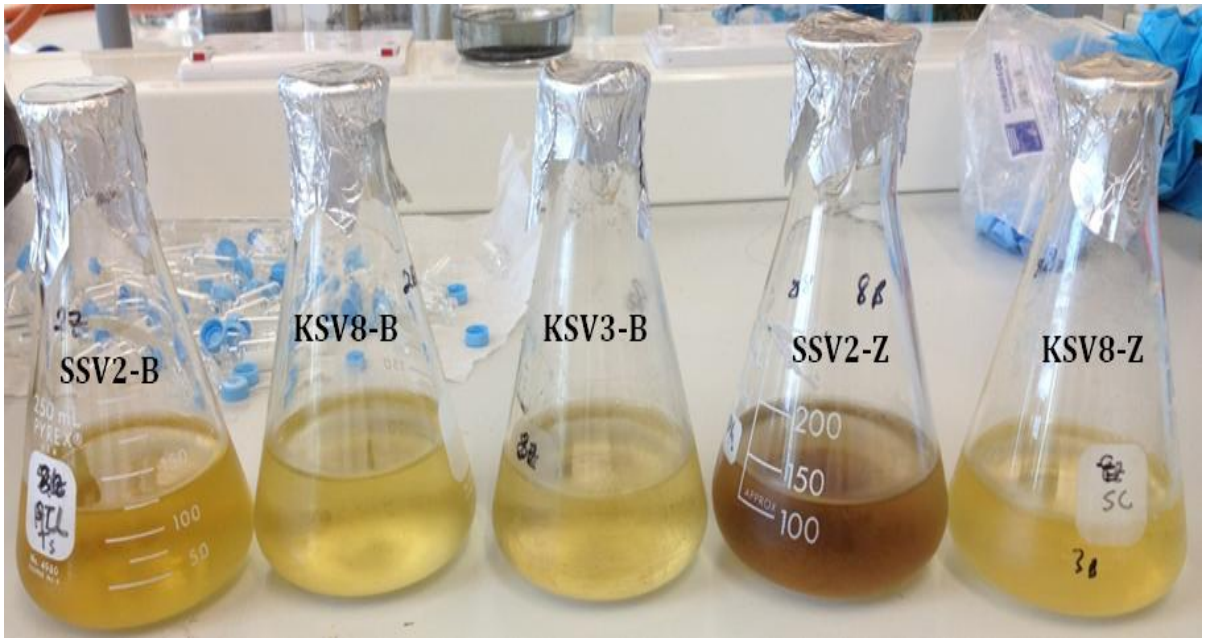


Fig 4.6 Sorghum bagasse over-limed hydrolysate after charcoal filtration. SSV2, KSV8 and KSV3 sorghum bagasse cultivated in Kano and Kaduna, Nigeria. These are bagasse hydrolysates after detoxification by overliming with calcium hydroxide crystals and charcoal filtration.

Importantly, the final hydrolysates after detoxification contained over 150 mg/L of FAN level (Table 4.4) that is necessary for efficient fermentation process (Thomas and Ingledew, 1990). Also, observed amino acids concentrations for hydrolysates

showed that the most essential amino acids required by yeast cells for efficient metabolism during fermentations were present in all the hydrolysates (Table 4.6). it is expedient to mention that because of specificity and sensitivity of the K-LARGE/K-PANOPA assay kits applications, certain amino acids were not detected by these kits (Megazyme, Northern Ireland), hence, the total amino acids concentration of the hydrolysates appeared to be higher than that of corresponding FAN levels, instead of the other way round since the amino acid is only a component of FAN composition. With regards to the hydrolysates amino acids profiles, most of the important Group 1 amino acids that cannot be biosynthesized by yeast cells are available in the SSV2, KSV8 and KSV3 hydrolysates (Table 4.6), these Group 1 amino acids are important because they are normally assimilated by yeasts at onset of fermentation (Walker, 1998; Boulton and Quain, 2001). Furthermore, the Group 2 and other Group amino acids that are normally sequentially assimilated by yeasts during the course of fermentation progress are also available in the hydrolysates, even though these groups of amino acids may be synthesized by yeasts during the course of fermentation (Lekkas *et. al*., 2007; Feldmann, 2012).

Table 4.6 Initial amino acids of charcoal filtered hydrolysates (µmol/mL)

Item	Kano			Kaduna	
Amino acid	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Group 1					
aspartic	1.492^{a} ±0.001	0.509^{b} ±0.006	0.753^{c} ±0.002	0.618^{d} ±0.004	1.279^{e} ±0.010
glutamic	0.240^{a} ±0.003	0.085^{b} ±0.007	0.176^{c} ±0.024	0.186^{c}±0.007	0.221^{d} ±0.005
serine	0.234^{a} ±0.001	0.118^{d} ±0.008	0.135^{c} ±0.018	0.095^{e} ±0.005	0.216^{b} ±0.007
arginine	0.099^{a} ±0.001	0.027^{c} ±0.004	0.041^{c} ±0.011	0.025^{c} ±0.005	0.072^{b} ±0.005
threonine	0.157^{a} ±0.002	0.055^{d} ±0.007	0.091^{c} ±0.013	0.061^{d} ±0.005	0.123^{b} ±0.005
lysine	0.113^{a} ±0.001	0.020^{b} ±0.003	0.051^{c} ±0.014	0.036^{d} ±0.005	0.092^{e} ±0.005
asparagine	*ND	*ND	*ND	*ND	*ND
glutamine	*ND	*ND	*ND	*ND	*ND
Sub-total	***2.330 ±0.002***	***0.813 ±0.037***	***1.241* ±0.021**	***1.019 ±0.033***	***2.000 ±0.006***
Group 2					
methionine	0.206^{a} ±0.002	0.081^{d} ±0.002	0.104^{c} ±0.010	0.086^{d} ±0.004	0.186^{b} ±0.006
Valine	0.237^{a} ±0.001	0.095^{b} ±0.008	0.117^{b} ±0.011	0.102^{c} ±0.005	0.222^{d} ±0.008
isoleucine	0.110^{a} ±0.001	0.029^{b} ±0.006	0.052^{c} ±0.007	0.040^{c} ±0.004	0.094^{d} ±0.006
leucine	0.350^{a} ±0.000	0.067^{b} ±0.003	0.138^{c} ±0.002	0.118^{d} ±0.003	0.226^{e} ±0.005
phenylalanine	0.061^{a} ±0.002	0.016^{b} ±0.004	0.027^{b} ±0.013	0.029^{b} ±0.007	0.050^{a} ±0.006
histidine	0.077^{a} ±0.001	0.031^{e} ±0.001	0.040^{b} ±0.016	0.026^{c} ±0.002	0.075^{a} ±0.006
Sub-total	***1.039 ±0.003***	***0.319 ±0.003***	***0.477* ±0.020**	***0.400 ±0.009***	***0.853 ±0.015***
Other groups					
glycine	0.335^{a} ±0.004	0.215^{b} ±0.008	0.174^{c} ±0.012	0.154^{d} ±0.006	0.254^{e} ±0.006
alanine	1.045^{a} ±0.003	0.279^{b} ±0.008	0.473^{c} ±0.076	0.343^{d} ±0.005	0.889^{e} ±0.008
proline	0.335^{a} ±0.001	0.114^{b} ±0.008	0.149^{c} ±0.015	0.105^{b} ±0.006	0.271^{d} ±0.007
tyrosine	0.104^{a} ±0.003	0.090a ±0.004	0.065^{b} ±0.010	0.173^{d} ±0.004	0.072^{b} ±0.005
tryptophan	*ND	*ND	*ND	*ND	*ND
Sub-total	***1.818 ±0.003***	***0.698 ±0.004***	***0.860* ±0.054**	***0.775 ±0.009***	***1.485 ±0.014***
Grand Total	**5.186^{a} ±0.008**	**1.829^{b} ±0.044**	**2.577^{c} ±0.095**	**2.1925^{d} ±0.05**	**4.338^{e} ±0.035**

SSV2, KSV8 and KSV3 sorghum bagasse comprising crushed stalks, leaves, peduncles and panicles were cultivated in Kano and Kaduna (Nigeria) hydrolysed by dilute H_2SO_4 acid followed by enzymatic saccharification. The hydrolysates were overlimed with $Ca(OH)_2$ and filtered with charcoal. The Amino acids were determined by GC-MS. Means on the same row that do not share same superscript letter (a-e) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected.

Finally, several previous studies that investigated various pre-treatment options for sorghum bagasse have reported varied fermentable sugar yields, our results in this study compared favourably with most of the results briefly summarised in Table 4.7.

Table 4.7 Comparison of this study bagasse sugar yields to previous literature.

Sorghum pretreatment method	Sugar yields (g/100g substrate)	Reference
2% (v/v) H_2SO_4 digestion at 75^oC for 2 h followed by 24 h enzymatic hydrolysis	24-47 g glucose & 17-20 g xylose.	This study
3% CaOH digestion at 121^oC for 1 h followed by 24 h enzymatic hydrolysis.	40 g glucose & 21 g xylose	Kim *et al*. (2012)
Microwave assisted ammonium hydroxide digestion at 130^oC for 1 h	42 g glucose	Chen *et al*. (2012)
10% (w/w) NaOH digestion at 70^oC for 4 h followed by 24 h enzymatic hydrolysis.	31 g glucose & 14 g xylose	Panagiotopoulos *et al.* (2010)
3% H_2SO_4 digestion for 10 min followed by 96 h enzymatic hydrolysis.	37 g glucose & 21 g xylose	Phuengjayaem and Teeradakorn (2011)
10%(w/v) NaOH at 121^oC for 25 min followed by 21% (v/v) H_2SO_4, digestion at 70^oC for 73 min	21 g glucose	Thanapimmetha *et al.* (2011)
2% NaOH digestion followed by 24 h enzymatic hydrolysis	26 g glucose	Sathesh-Prabu and Murugesan (2011)
Ammonium fibre explosion (AFEX) at 140^oC for 30 min followed by 72 h enzymatic hydrolysis	29 g glucose & 15 g xylose	Li *et al.* (2010)

4.3.3. Fermentation performances.

While in this study *P. tannophilus* is considered a xylose fermenting yeast, the *S. cerevisiae* cells were glucose fermenting yeasts only. The fermentation performance of these yeasts would depend on the fermentation media condition such as pH, temperature, fermentation media sugar and FAN concentrations as well as alcohol tolerance level of the cells (Olsson and Hahn-Hagerdal, 1996; Sathesh-Prabu and Murugesan, 2011). Therefore, for effective comparison of fermentation performance of SSV2, KSV8 and KSV3 hydrolysates in this study, *P. tannophilus* and *S. cerevisiae* yeasts were selected out of five different yeast strains previously investigated (not reported here).

1. Enzymatic hydrolysates fermentation.

The non-detoxified or crude enzymatic hydrolysates are considered to contain acetic acids, furan derivatives and phenols all of which were the by-products of acid hydrolysis of bagasse. Combinations of these compounds in fermentation hydrolysates inhibits efficient yeast metabolism which may result in stuck fermentation depending on the concentration level of the inhibitory compounds present in the fermentation substrates (Olsson and Hahn-Hagerdal, 1996; Zacchi, 2011; Cao *et al.,* 2012). The *S. cerevisiae* and *P. tannophilus* yeasts show notable lag time within initial 24 h of fermentation progress; this yeast lag phase represents the period that the yeast cells take to get adapted to the fermentation media (Kurian *et al.,* 2010). Therefore, yeast cells will tend to take longer lag time in harsh media than in nutrient rich media, consequently, the inhibitory compounds concentrations in SSV2, KSV8 and KSV3 hydrolysates significantly contributed to the observed yeast lag phase of these hydrolysates (Figs. 4.7 and 4.8). The *S. cerevisiae* cells showed shorter lag time (Fig. 4.7) and generally appeared to exhibit better tolerance level in the media than *P. tannophilus* (Fig. 4.8) (Sathesh-Prabu and Murugesan, 2011). However, as fermentation progresses into later stage, *P. tannophilus* that is able to ferment pentose sugars showed overall higher observed CO_2 gas production than *S. cerevisiae*. The SSV2B substrate appeared a most favourable substrate followed by KSV3B. Although *S. cerevisaie* showed faster fermentative capacity with SSV2B substrates, the *P. tannophilus* showed overall improved higher ethanol yields at end of the fermentation process (Figs. 4.9 and 4.10). The observed *S. cerevisiae* kinetics reported in this study compares with kinetics profile reported by Han *et al.,* (2012), who reported similar kinetics for sorghum bagasse hydrolysates (that is non-

detoxified) and fermented by *S. cerevisiae* in which the yeasts took about 60 h to reached maximum CO_2 gas production.

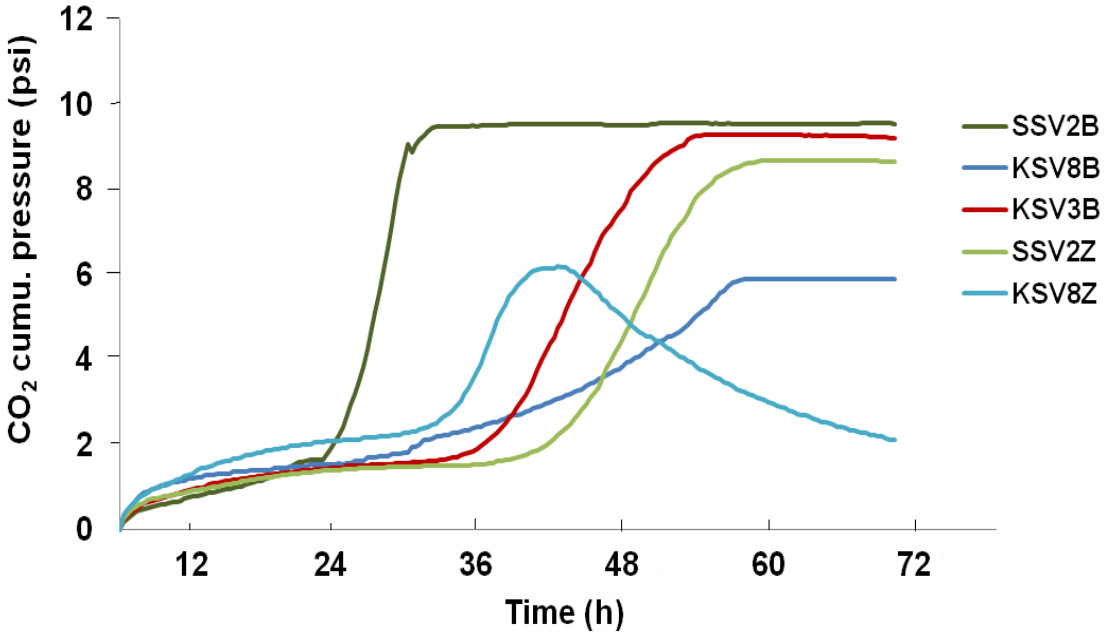


Fig. 4.7 *S. cerevisiae* fermented enzymatic hydrolysates kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse cultivated at sites B and Z (Nigeria). Fermentation was monitored by observed rate of CO_2 gas formation via ANKOM^{RF} system.

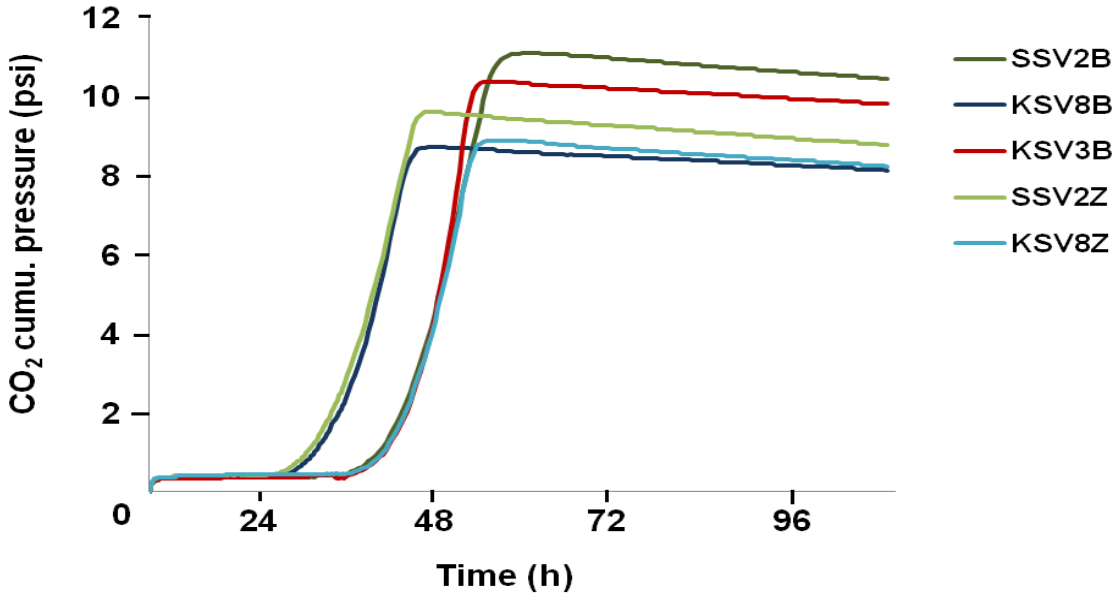


Fig 4.8 *P. tannophilus* fermented enzymatic hydrolysates kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse cultivated at sites B and Z (Nigeria). Fermentation was monitored by observed rate of CO_2 gas formation via ANKOM^{RF} system.

With regards to ethanol production, the *P. tannophilus* show higher corresponding ethanol yields than *S. Cerevisiae* cells (Figs. 4.9 and 4.10). These results were consistent with the fermentation kinetics results. The SSV2B and KSV3B substrates showed similar observed ethanol yields both for the *S. cerevisiae* and *P. tannophilus* cells respectively (Figs. 4.9 and 4.10). However, these fermentation processes may be considered inefficient in terms of level of sugar utilisation; the total observed fermentable sugar utilisation ranged between 32-54% while the alcohol yields were less than 40% of theoretical yields (Tables 4.8 to 4.10). Consequently, the combined effects of inhibitory compounds initially present in the media along with additional acetaldehydes, aldehydes among other toxic compounds produced during fermentation renders the fermentative capacity of yeasts inefficient in the media (Navarro *et al*., 2012). Nevertheless, the maximum observed ethanol yields of SSV2B substrate (12-13 g/L) fermented by both *S. cerevisiae* and *P. tannophilus* compares favourably with 14 g/L ethanol yield reported by Ban *et al*. (2008) for sorghum bagasse pre-treated with phosphoric acid (80 g/L H_3PO_4) at 120^oC for 80 min and fermented by *P. tannophilus.* Furthermore, the reported ethanol yields of 7-11 g/L for KSV8 and KSV3 substrates in this study compares well with 5 g/L ethanol concentration reported in previous literature for sorghum bagasse fermented by *S. cerevisiae* (after phosphoric acid pre-treatment) and 6 g/L ethanol yield from sorghum bagasse hydrolysates pre-treated with dilute $NaOH/H_2O_2$ solutions and fermented by *S. cerevisiae* (Ban *et al.,* 2008; Cao *et al.,* 2012).

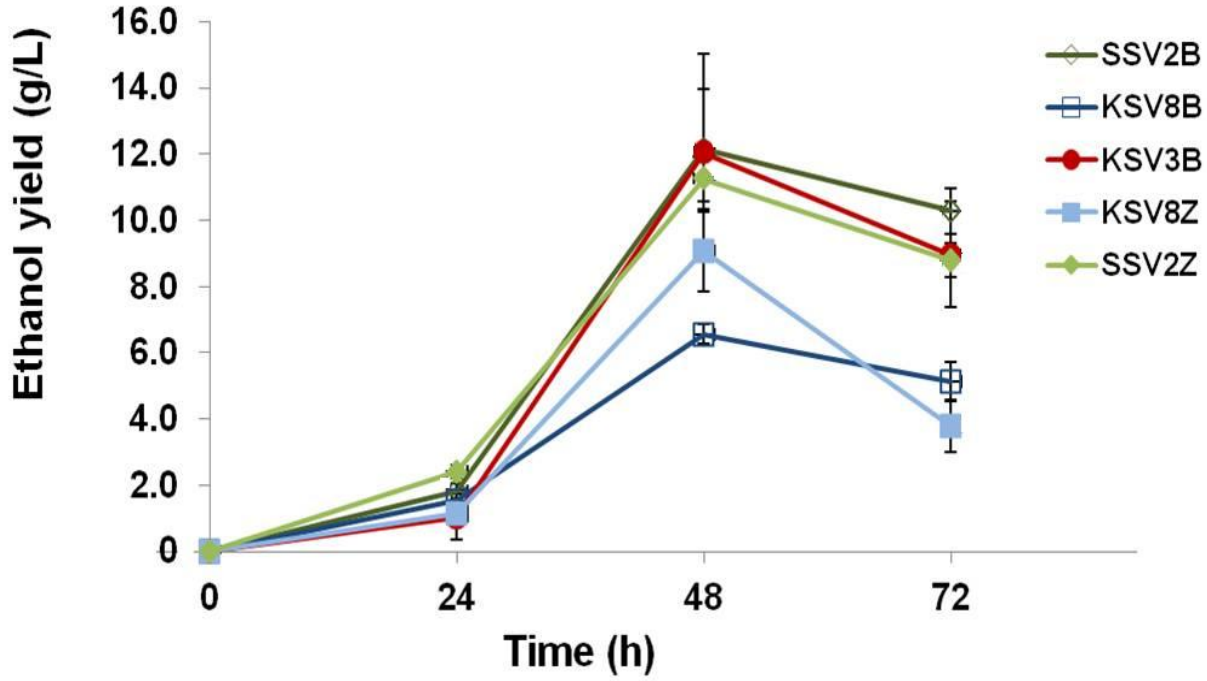


Fig 4.9 *S. cerevisiae* fermentation profile with enzymatic hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32^{o}C and 120 rpm orbital shaking. Samples were withdrawn after every 24h for ethanol determination (FermentoFlash®, Germany). Results are std. means of duplicate experiments.

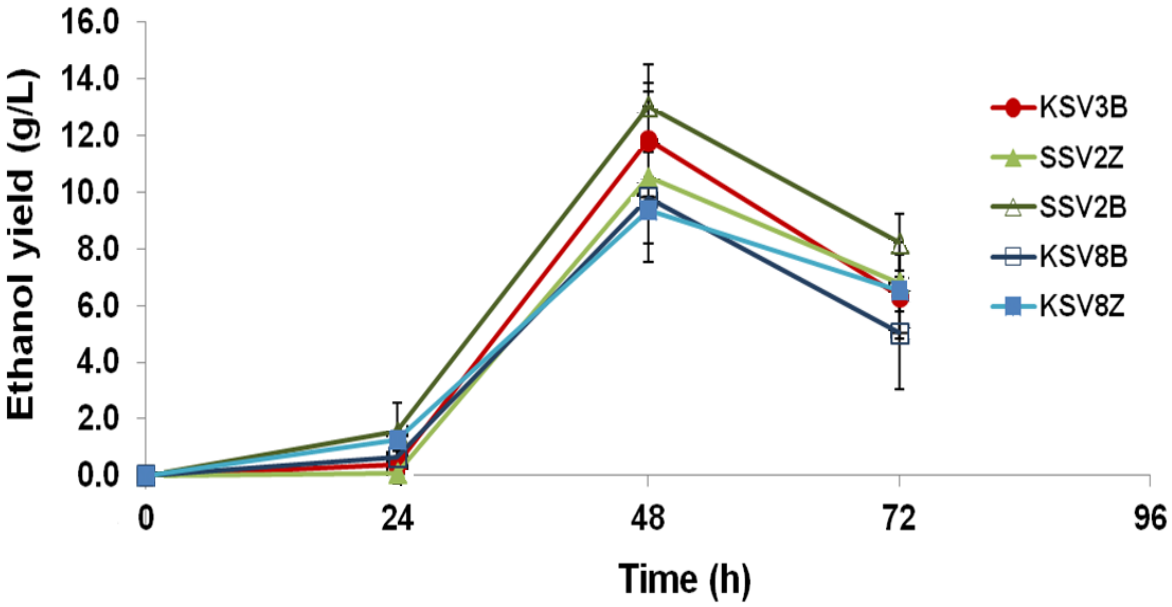


Fig 4.10 *P. tannophilus* fermentation profile with enzymatic hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32^{o}C and 120 rpm orbital shaking. Samples were withdrawn after every 24 h for ethanol determination (FermentoFlash®, Germany). Results are std. means of duplicate experiments.

2. Over-limed hydrolysates fermentation.

Largely as a result of the removal of aliphatic and organic acids from SSV2, KSV8 and KSV3 sorghum bagasse hydrolysates by over-liming (Heredia-Olea *et al.,* 2013). However, due to the presence of phenols along with residual aliphatic acids likely present in the over-limed hydrolysates, yeasts would still exhibit observable lag phase (Frosch *et al.,* 2008; Cao *et al.,* 2012). *P. tannophilus* show improved lag time and fermentation kinetics than *S. cerevisiae* (Figs. 4.11-12). This suggested the over-limed hydrolysates contained less toxic compounds; therefore, the yeasts appeared to function better when compared to the crude enzymatic hydrolysates. For example, while *P. tannophilus* reached near maximum CO_2 production under 24 h from start of fermentation, the *S. cerevisiae* took about 36 h. However, the observed faster fermentation kinetics of *P. tannophilus* cells relative to *S. cerevisiae* is not clearly understood yet but it is thought to be related to the ability of the former to effectively metabolise pentose sugars (which are additional source of sugars) compared to the latter cells that can only ferment hexose sugars (Olsson and Hahn-Hagerdal, 1996). SSV2B substrate followed by KSV3B appeared to be the most favourable fermentation substrates in the context of final CO_2 gas production volume while SSV2Z substrates may be favoured in terms of faster fermentation kinetics.

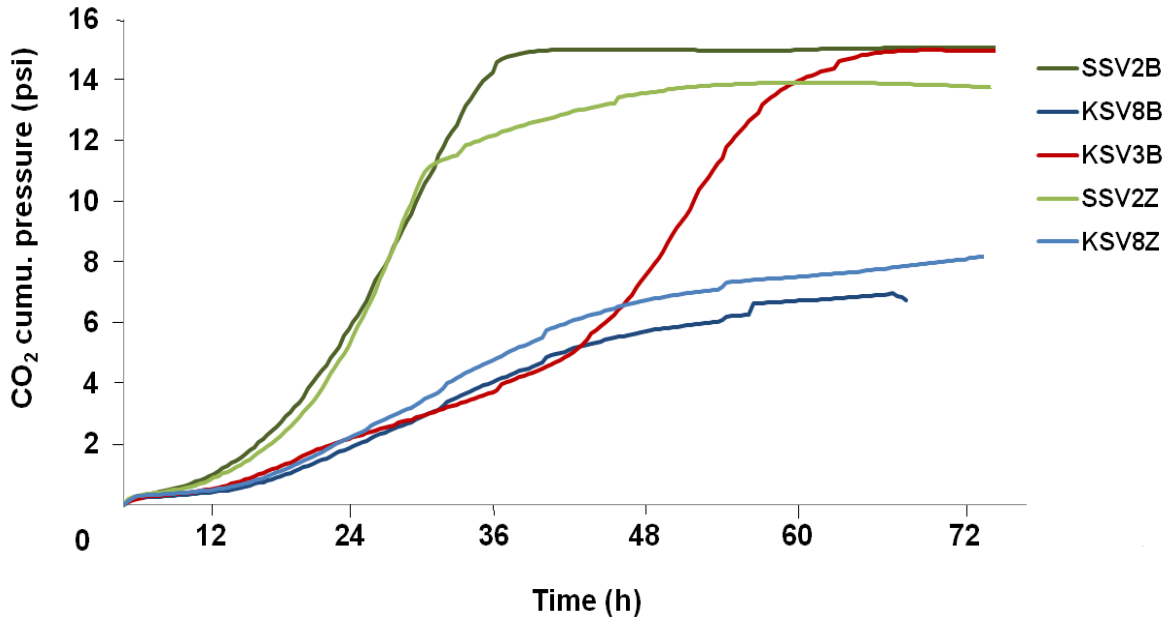


Fig. 4.11 *S. cerevisiae* fermented over-limed hydrolysates kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse overlimed hydrolysates. The fermentation progress was monitored by rate of CO_2 gas formation via ANKOM^{RF} system.

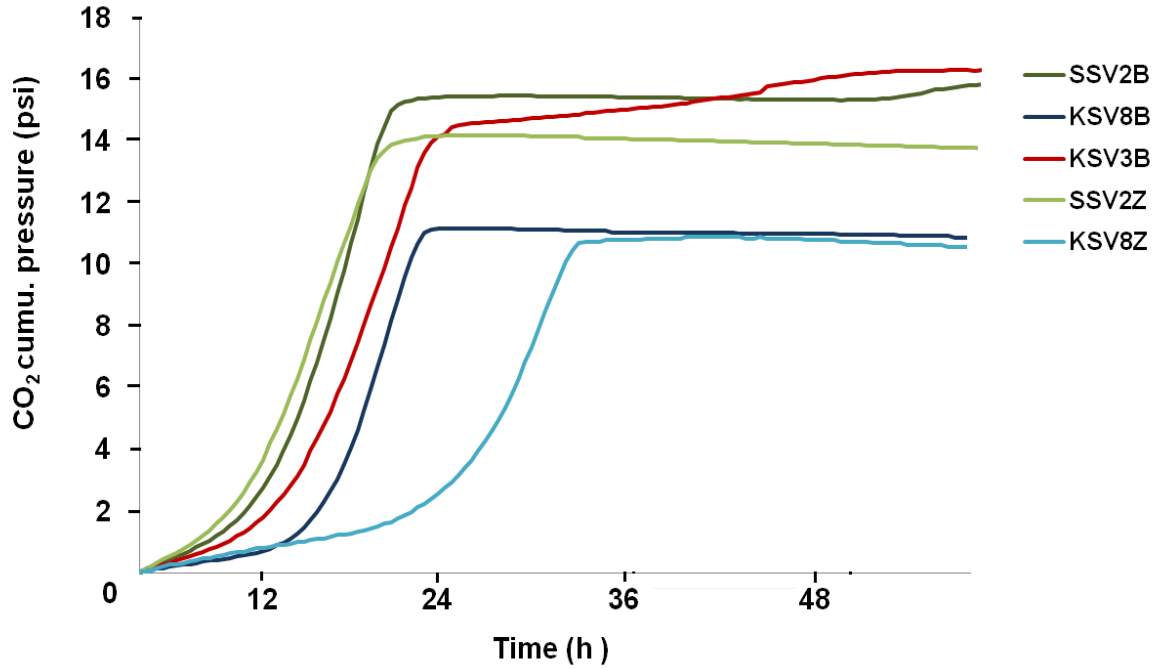


Fig 4.12 *P. tannophilus* fermented over-limed hydrolysates kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse overlimed hydrolysates. The fermentation progress was monitored by rate of CO_2 gas formation via ANKOM^{RF} system.

In terms of observed ethanol production, *P. tannophilus* consistently showed improved performance over *S. cerevisiae*, this may arguably be due to the ability of the former to metabolise a wider range of sugars in the media than the latter. For example, while the former showed near maximum ethanol concentration within 24 h of fermentation commencement time (Fig. 4.13), the latter took about 48 h to reach near maximum ethanol concentration level (Fig. 4.14). Furthermore, the fermentation efficiency for these substrates appeared better than the previous substrates, for example, the observed sugar utilisation for *P. tannophilus* cells has improved from the previous 32-54% to now 52-69% range while that of *S. cerevisiae* cells has also increased to 46-61% range respectively. But despite this notable improvement in ethanol yields and CO_2 production as well as the increased sugar utilisation capacity of the yeast cells, there is still room for improvement of the cells fermentation performances because residual sugars were observed in the fermented broth, particularly for SSV2 and KSV3 substrates (Tables 4.8 and 4.9). Nevertheless, the observed reported ethanol yield of about 17 g/L for both SSV2B and KSV3B substrates fermented by *P. tannophilus* and *S. cerevisiae* cells respectively (Figs. 4.13-14) compares favourably to ethanol yields of 16-19 g/L (after 96 h fermentation time) reported by Wan *et al.,* (2012) and Cao *et al*. (2012) for sorghum bagasse hydrolysate fermented by co-culture of (*S. cerevisiae* **-** *P. Stipitis*) and by *S. cerevisiae* cells respectively.

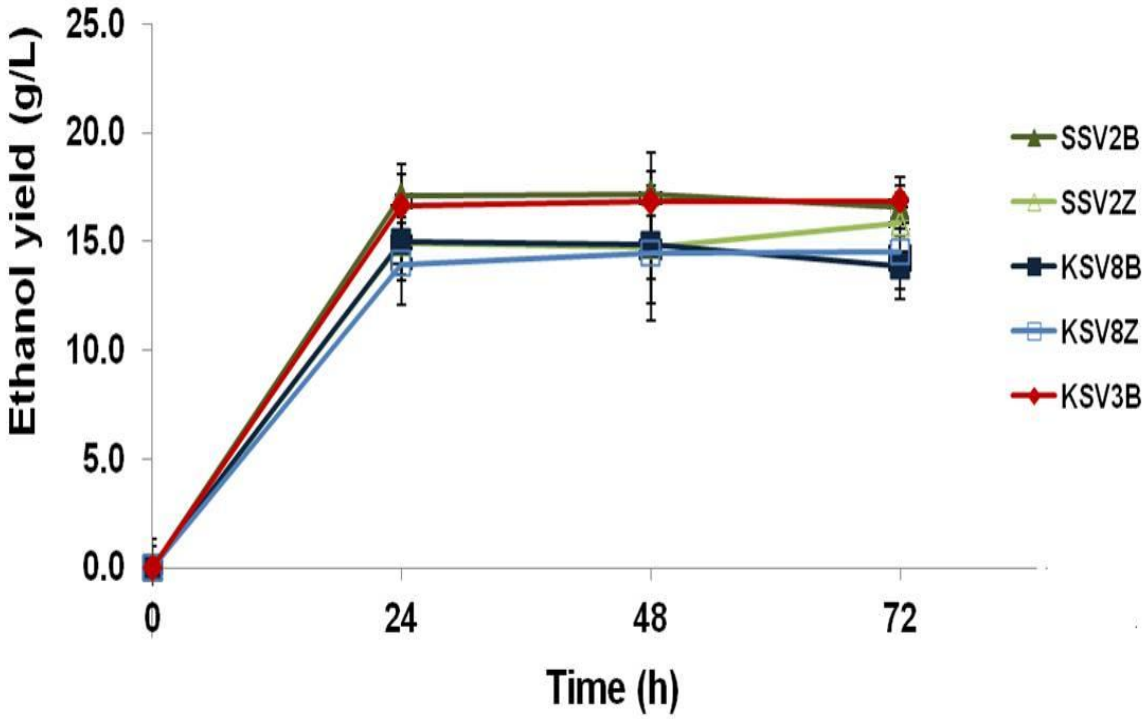


Fig. 4.13 *P. tannophilus* fermentation profile with over-limed hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32°C and 120 rpm orbital shaking. Samples were withdrawn after every 24 h for ethanol determination (FermentoFlash®, Germany). Std. means of duplicate experiments.

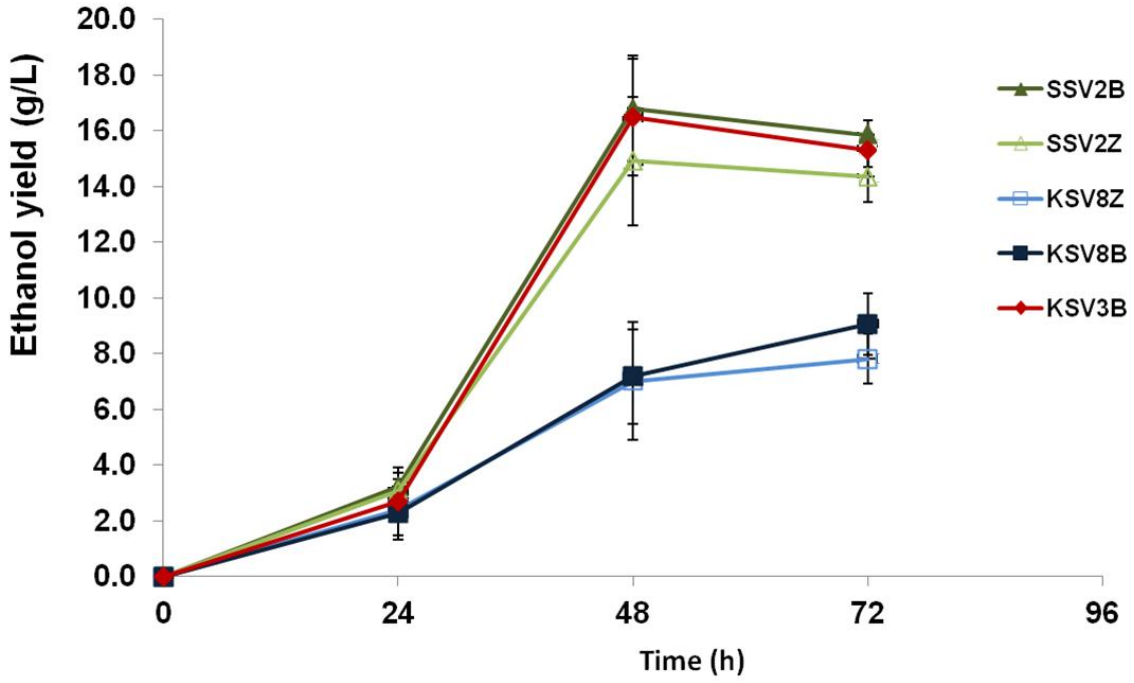


Fig. 4.14 *S. cerevisiae* fermentation profile with over-limed hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32°C and 120 rpm orbital shaking. Samples were withdrawn after every 24 h for ethanol determination (FermentoFlash®, Germany). Results are std. means of duplicate experiments.

3. Charcoal treated hydrolysates fermentation

Polyphenols are by-products of lignin degradation and hinder effective yeast metabolism during fermentation (Ge, *et al.,* 2011; Zacchi, 2011). Therefore, removal of phenolic compounds by charcoal filtration from SSV2, KSV8 and KSV3 hydrolysates resulted in a drastic reduction in yeast lag phase with resultant significant increase in fermentation rates of both *P. tannophilus* and *S. cerevisiae* yeast cells respectively (Figs. 4.15-16), although the former show faster observed fermentation rates than the latter. While *P. tannophilus* yeast approached near maximum cell growth rates at about 12 h after start of fermentation, *S. cerevisiae* cells took about 24 h in terms of CO_2 gas formation rates. These reported fermentation kinetics are similar to the kinetics reported by Nichols *et. al*. (2010) for charcoal filtered sorghum bagasse hydrolysates fermented by *S. cerevisiae* yeast under CO_2 gas production monitoring. However, Gyalai-Korpos *et al.* (2008) reported faster fermentation rates for detoxified sorghum bagasse hydrolysates (supplemented with exogenous yeast nutrients) and fermented by *S. cerevisiae* yeast, they reported achieving maximum CO_2 gas production about 4 h into start of fermentation. Finally, SSV2B followed by KSV3B hydrolysates apparently appeared to be most favourable substrates under both the *P. tannophilus* and *S. cerevisiae* fermentation conditions (Figs. 4.15-16).

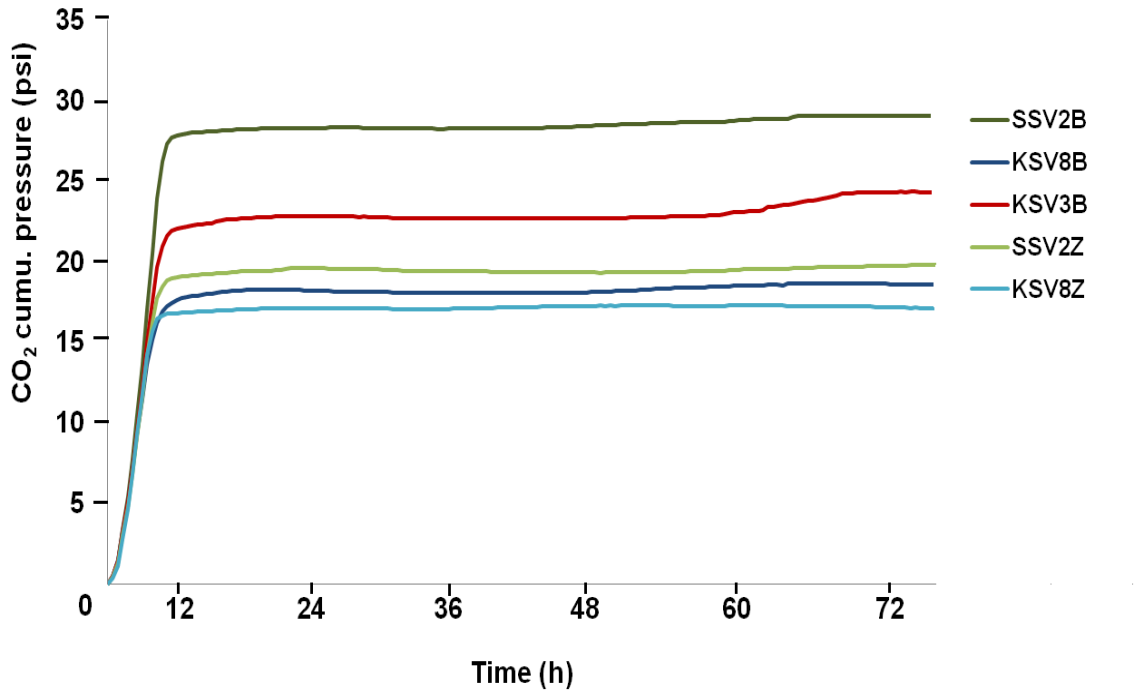


Fig 4.15 *P. tannophilus* fermented charcoal filtered hydrolysate kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse charcoal filtered hydrolysates. The fermentation progress was monitored by rate of CO_2 gas formation via ANKOM^{RF} system.

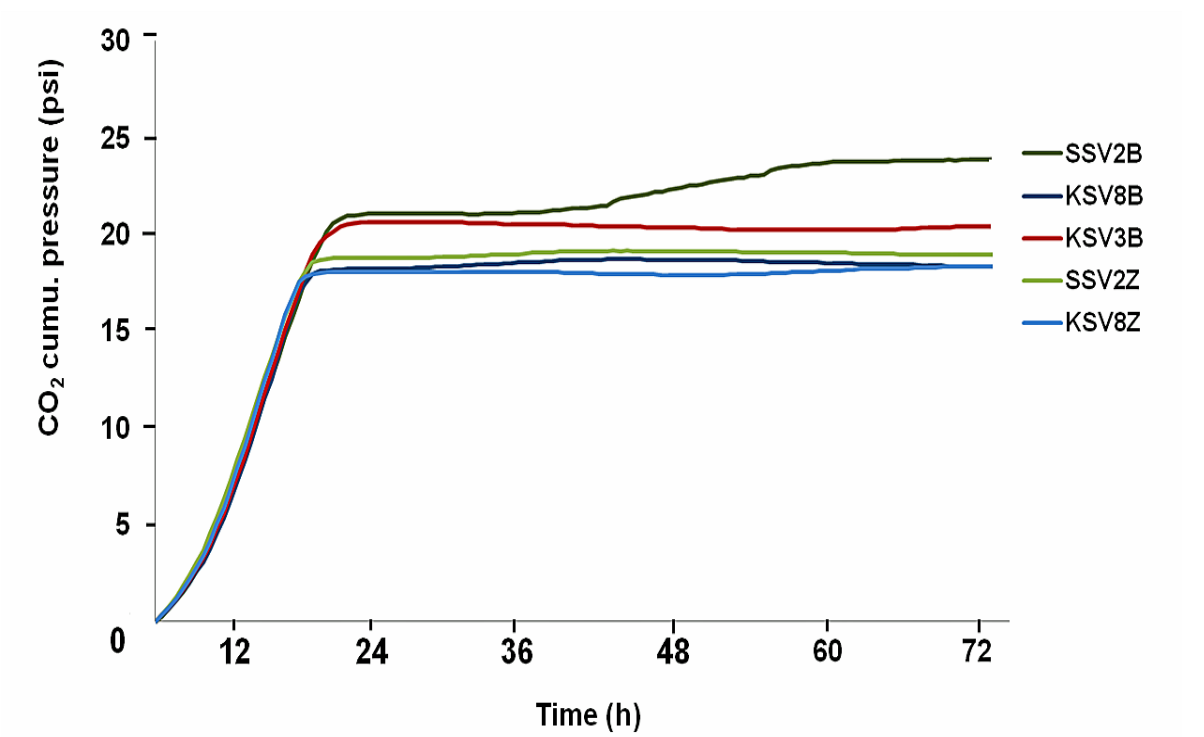


Fig. 4.16 *S. cerevisiae* fermented charcoal filtered hydrolysate kinetics. Fermentation kinetics of SSV2, KSV8 and KSV3 sorghum bagasse charcoal filtered hydrolysates. The fermentation progress was monitored by rate of CO_2 gas formation via ANKOM^{RF} system.

With regards to observed ethanol production, SSV2B followed by KSV3B are apparently the most favourable fermentation substrates (Figs. 4.17-18). The *P. tannophilus* cells showed a more favourable ethanol yields over *S. cerevisiae* cells perhaps because of ability of the former to metabolise pentose sugars over the latter. The observed sugar utilisation of *S. cerevisiae* ranged between 59-76% and that of *P. tannophilus* 75-81% for SSV2, KSV8 and KSV3 bagasse substrates respectively. These results were notable improvements over the sugar utilisation capacity previously achieved with the over-limed hydrolysates of SSV2, KSV8 and KSV3 sorghum bagasse. However, in spite of *P. tannophilus* cells observed higher sugar utilisation during fermentation, its final ethanol yields were mostly similar to those of *S. cerevisiae* cells, comparing similar fermentation substrates, however, the corresponding CO_2 gas production rates were higher for the *P. tannophius* cells than for *S. cerevisiae* (Table 4.10). Consequently, it is observed that that *P. tannophilus* yeast have higher fermentable sugar utilisation than S. Cerevisiae but produces almost similar ethanol yield as the *S. cerevisiae* cells but with higher CO_2 gas formation. These may perhaps be due to *P. tannophilus* being a xylose fermenting yeast and would normally require xylose concentration in the media to be in the range of 5-8 g/L (under low glucose concentration) for the cells to efficiently ferment the xylose to ethanol (Olsson and Hahn-Hagerdal, 1996). However, when the xylose concentration is above 8 g/L and the glucose concentration is relatively high as well in the starting fermentation media just as is the case for SSV2, KSV8 and KSV3 hydrolysates, there is the tendency that *P. tannophilus* cells would more or less direct its xylose metabolism mechanism more of towards xylitol production rather than alcohol (Slininger *et al.,* 1990; Liang *et al*., 2010). This condition may possibly explain the consistently observed higher CO_2 gas production with corresponding

lower than would be expected ethanol production from *P. tannophilus* cells relative to the *S. cerevisiae*.

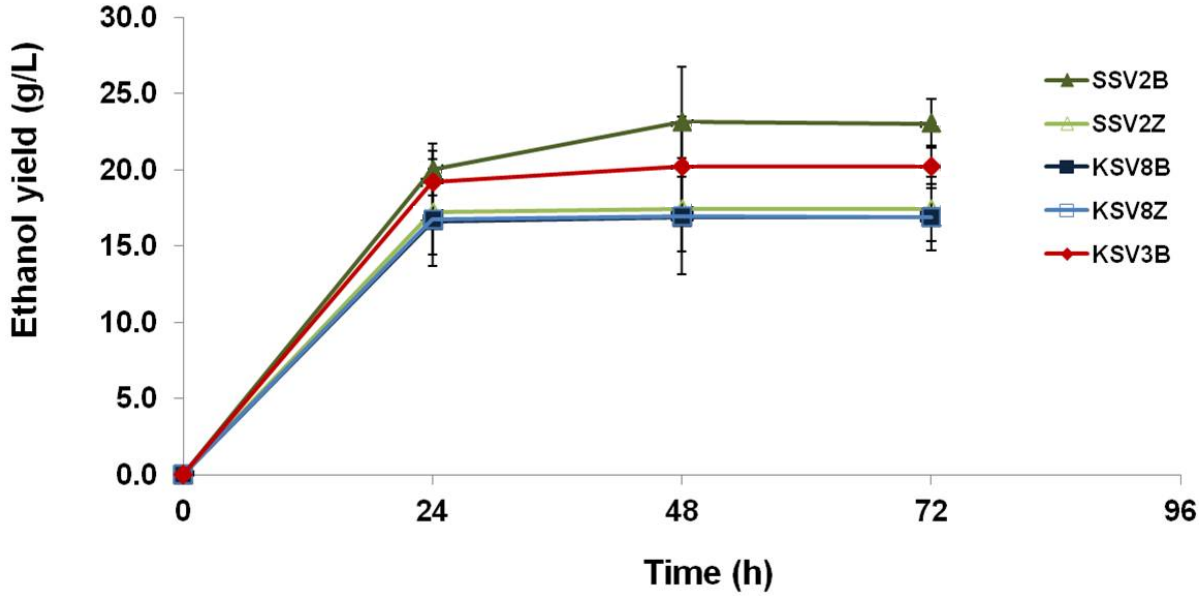


Fig. 4.17 *P. tannophilus* fermentation profile with charcoal filtered hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32^{o}C and 120 rpm orbital shaking. Samples were withdrawn after every 24 h for ethanol determination (FermentoFlash®, Germany). Results are Std. means of duplicate experiments.

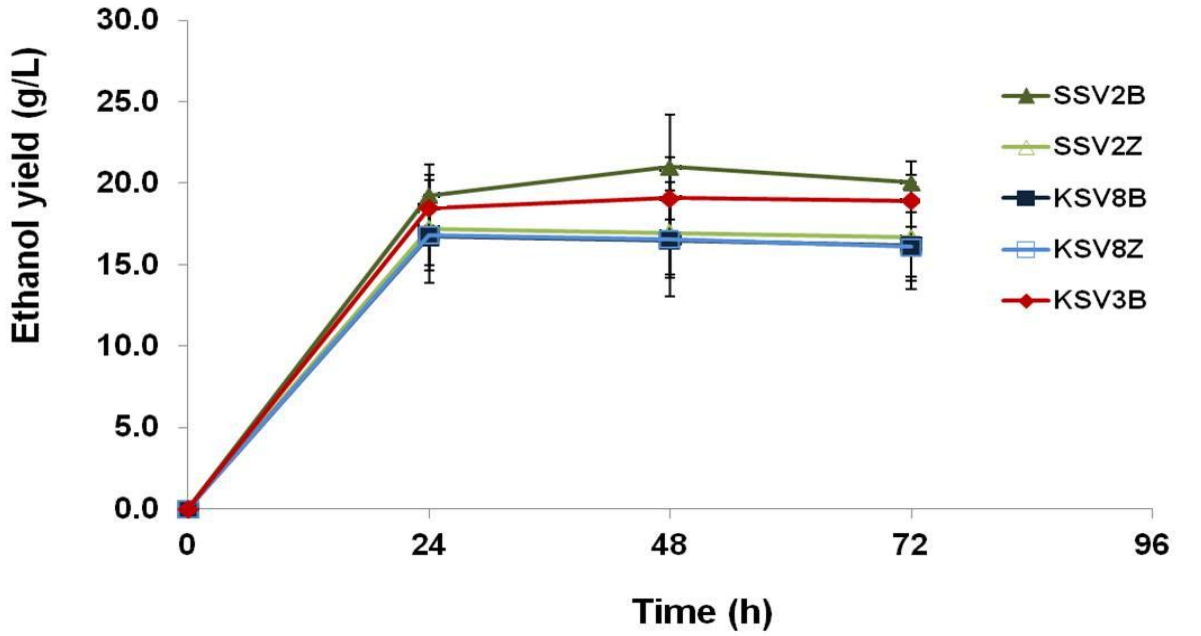


Fig. 4.18 *S. cerevisiae* fermentation profile with charcoal filtered hydrolysates as substrates. SSV2, KSV8 and KSV3 bagasse hydrolysates fermented at 32^{o}C and 120 rpm orbital shaking. Samples were withdrawn after every 24 h for ethanol determination (FermentoFlash®, Germany). Results are Std. means of duplicate experiments.

Table 4.8 *P. tannophilus* fermentation residual sugars (g/100g bagasse)

Bagasse	Hydrolysates	Glucose	Xylose	Arabinose	Total sugars
	Enzymatic	13.25^{a} ±0.2	13.71^{a} ±0.5	4.93^{a} ±0.5	31.89^{a} ±1.2
SSV2B	$Ca(OH)_2$ Overlimed	2.89^{d} ±0.9	12.57^{a} ±1.1	4.46^{a} ±0.4	19.92^{b} ±0.6
	Charcoal filtrate	*ND	8.76^{bc} ±0.9	3.65^{b} ±0.3	12.41^{c} ±1.1
	Enzymatic	14.17^{a} ±2.0	14.70^{c} ±1.1	4.58^{a} ±0.1	33.45^{d} ±1.0
SSV2Z	$Ca(OH)_2$ Overlimed	2.87^{d} ±0.5	12.56^{a} ±1.5	4.46^{a} ±0.9	19.89^{b} ±1.1
	Charcoal filtrate	*ND	11.08^{d} ±0.2	3.21^{b} ±0.1	14.29^{e} ±0.2
	Enzymatic	10.42^{b} ±1.2	17.67^{e} ±1.1	5.49^{c} ±0.4	33.58^{d} ±1.9
KSV8B	$Ca(OH)_2$ Overlimed	*ND	14.51^{c} ±0.9	5.86^{c} ±0.1	20.37^{b} ±0.9
	Charcoal filtrate	*ND	7.30^{b} ±1.2	3.01^{d} ±0.2	10.31^{f} ±1.3
	Enzymatic	11.15^{b} ±0.8	18.04^{e} ±1.7	4.74^{a} ±0.3	33.93^{d} ±1.2
KSV8Z	$Ca(OH)_2$ Overlimed	*ND	15.85^{f} ±0.4	4.52^{a} ±0.6	20.51^{b} ±0.9
	Charcoal filtrate	*ND	7.45^{b} ±0.7	2.88^{e} ±0.1	10.33^{f} ±0.8
	Enzymatic	9.18^{c} ±1.2	16.14^{f} ±1.1	3.94^{b} ±0.5	29.26^{ab} ±1.7
KSV3B	$Ca(OH)_2$ Overlimed	*ND	14.86^{c} ±0.3	4.72^{a} ±0.7	20.08^{b} ±1.0
	Charcoal filtrate	*ND	9.45^{bc} ±0.6	3.08^{d} ±0.1	12.53^{c} ±0.8

Residual sugars in sorghum bagasse hydrolysates after 72 h fermentation by *P. tannophilus* without exogenous nutrient supplementation and the sugars were determined by HPLC. Corresponding Means in the same column that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected.

Table 4.9 *S. cerevisiae* fermentation residual sugars (g/100g bagasse)

Bagasse	Hydrolysates	Glucose	Xylose	Arabinose	Total sugars
	Enzymatic	16.32^{a} ±1.2	16.33^{a} ±0.3	5.06^{b} ±0.5	37.71^{a} ±2.0
SSV2B	$Ca(OH)_2$ Overlimed	9.40^{b} ±0.7	13.88^{b} ±1.2	4.63^{a} ±0.5	27.91^{b} ±1.3
	Charcoal filtrate	*ND	11.20^{c} ±1.1	4.71^{a} ±0.8	15.91^{c} ±1.9
	Enzymatic	16.57^{a} ±1.7	15.89^{a} ±1.1	4.97^{b} ±0.1	37.43^{a} ±1.0
SSV2Z	$Ca(OH)_2$ Overlimed	6.69^{c} ±0.6	12.46^{b} ±0.9	4.45^{a} ±0.8	23.60^{d} ±2.3
	Charcoal filtrate	*ND	10.08^{c} ±0.6	3.89^{c} ±0.3	13.97^{e} ±1.0
	Enzymatic	9.58^{b} ±1.2	18.93^{d} ±1.8	6.29^{d} ±0.5	34.80^{f} ±2.5
KSV8B	$Ca(OH)_2$ Overlimed	*ND	19.01^{d} ±1.2	5.73^{e} ±0.4	24.74^{d} ±1.6
	Charcoal filtrate	*ND	12.21^{b} ±1.1	4.40^{a} ±0.3	16.61^{c} ±1.3
	Enzymatic	8.15^{b} ±1.1	18.21^{d} ±1.7	5.16^{b} ±0.6	31.52^{ab} ±2.1
KSV8Z	$Ca(OH)_2$ Overlimed	*ND	18.77^{d} ±0.9	5.16^{b} ±0.5	23.93^{d} ±1.4
	Charcoal filtrate	*ND	12.13^{b} ±1.1	4.68^{a} ±0.4	16.81^{c} ±1.4
	Enzymatic	17.42^{a} ±1.2	15.64^{a} ±0.9	5.04^{b} ±0.3	38.10^{a} ±0.6
KSV3B	$Ca(OH)_2$ Overlimed	7.04^{c} ±0.6	14.96^{e} ±1.3	5.04^{b} ±0.1	27.04^{b} ±1.8
	Charcoal filtrate	*ND	11.37^{c} ±1.0	4.79^{a} ±0.8	16.16^{c} ±1.8

Residual sugars in sorghum bagasse hydrolysates after 72 h fermentation by *S. cerevisiae*, sugars were determined by HPLC. Corresponding Means in the same column that do not share same superscript letter (a-f) are significantly different (p ≤0.05) by ANOVA using Turkey grouping method test. *ND = Not Detected.

Table 4.10 Fermentation ethanol and CO_2 yields

		P. tannophilus		*S. cerevisiae*	
Bagasse	**Hydrolysates**	**Ethanol** (g/L)	**CO_2 gas***	**Ethanol** (g/L)	**CO_2 gas***
SSV2B	Enzymatic	$13.03^{a} \pm 1.1$	$1423^{a} \pm 27$	$12.15^{a} \pm 0.88$	$1187^{a} \pm 23$
	$Ca(OH)_2$ Over-limed	$17.12^{d} \pm 0.9$	$2083^{b} \pm 31$	$16.81^{b} \pm 0.67$	$1930^{b} \pm 33$
	Charcoal filtrate	$23.12^{ad} \pm 0.5$	$3719^{c} \pm 24$	$20.99^{ff} \pm 0.94$	$3050^{c} \pm 26$
SSV2Z	Enzymatic	$10.53^{b} \pm 1.0$	$1237^{d} \pm 26$	$11.26^{a} \pm 0.98$	$1109^{d} \pm 19$
	$Ca(OH)_2$ Over-limed	$15.86^{e} \pm 0.4$	$1817^{e} \pm 22$	$14.91^{c} \pm 1.02$	$1783^{e} \pm 16$
	Charcoal filtrate	$17.44^{d} \pm 1.0$	2546 ± 21	$17.20^{d} \pm 0.96$	$2453^{ab} \pm 17$
KSV8B	Enzymatic	$9.81^{b} \pm 0.6$	$1142^{f} \pm 19$	$6.55^{e} \pm 0.59$	$754^{cd} \pm 22$
	$Ca(OH)_2$ Over-limed	$14.83^{f} \pm 0.8$	$1433^{a} \pm 23$	$8.60^{f} \pm 0.71$	$888^{ca} \pm 21$
	Charcoal filtrate	$16.89^{ab} \pm 0.3$	$2383^{ab} \pm 21$	$16.74^{b} \pm 0.48$	$2395^{da} \pm 25$
KSV8Z	Enzymatic	$9.36^{b} \pm 0.8$	$1125^{f} \pm 22$	$8.21^{f} \pm 0.69$	$793^{bc} \pm 33$
	$Ca(OH)_2$ Over-limed	$14.52^{f} \pm 0.3$	$1395^{ad} \pm 20$	$9.07^{f} \pm 0.77$	$1015^{db} \pm 18$
	Charcoal filtrate	$16.97^{ab} \pm 0.3$	$2217^{ae} \pm 22$	$16.81^{b} \pm 0.88$	$2314^{f} \pm 19$
KSV3B	Enzymatic	$11.84^{c} \pm 1.1$	$1382^{ef} \pm 31$	$12.05^{a} \pm 0.87$	$1124^{ef} \pm 21$
	$Ca(OH)_2$ Over-limed	$16.87^{ab} \pm 0.7$	$2093^{b} \pm 15$	$16.49^{b} \pm 0.64$	$1923^{df} \pm 20$
	Charcoal filtrate	$20.18^{ef} \pm 0.9$	$3118^{df} \pm 21$	19.11ff ± 0.91	$2647^{ae} \pm 23$

Ethanol and CO_2 gas yields of SSV2, KSV8 and KSV3 sorghum bagasse hydrolysates at three treatment levels. Fermentations were by *P. tannophilus* and S. cerevisiae yeasts (without exogenous nutrients supplementation). Corresponding Means in the same column that do not share same superscript letter (a-f) are significantly different ($p \leq 0.05$) by ANOVA using Turkey grouping method test. *****$C0_2$ gas (mL/100g dry bagasse).

Finally, Kano with warmer and drier climate appeared to be most favourable location for sorghum crop cultivation when the residue is destined for bioethanol production. Furthermore, the results reported here suggested SSV2 followed by KSV3 with corresponding ethanol yields of 292 L/t and 254 L/t (dry bagasse) at Kano are the favourable feedstock sources for bioethanol production, whilst KSV8 (Kano) had the least ethanol yield potential at 215 L/t dry bagasse. Several previous studies have reported varied ethanol yields from sorghum bagasse using various pre-treatment and fermentation conditions, our results compared favourably to previously reported literature results summarised in Table 4.11.

Table 4.11 Comparison of ethanol yields from this study to previous literatures

Fermentation condition	**Ethanol yield (g/L)**	**Reference**
Fermentation by *P. tannophilus* without nutrient supplementation.	17-23	This study
Fermentation by *S. cerevisiae* without nutrient supplementation.	16-20	This study
Fermentation by co-culture of *S. cerevisiae* and *Issatchenkia orientalis* and with nutrient supplements.	27	Wan *et al*. (2012)
Fermentation by *P. tannophilus* with nutrient supplements.	16	Ballesteros *et al.* (2003)
Fermentation by *S. cerevisiae* with nutrient supplementation.	23	Mehmood *et al.* (2009)
Simultaneous saccharification and fermentation (SSF) with *S. cereviciae* (5 g/L cell density) and nutrient supplementation	23	Shen *et al*. (2012)
Separate hydrolysis and fermentation (SHF) with *S. cereviciae* (3 g/L cell density) and nutrient supplementation	21	Shen *et al*. (2012)
Fermentation by co-culture of S. cerevisiae and *Neurospora crassa* with nutrient supplementation.	28	Dogaris *et al.* (2012)

4.4 Conclusion and recommendation.

The potentials for utilising whole sorghum crop residues in bioethanol production were investigated in this study. Our findings suggested that of the three Nigerian sorghum crops residues studied here, SSV2 followed by KSV3 sorghum residues are the most favourable feedstock sources for bioethanol production. Although SSV2 sorghum crop has the benefit of shorter cultivation duration and higher ethanol yield potential per unit biomass fermented, its relative lower total biomass yield per hectare crop cultivated makes it less competitive with KSV3 cultivar which has relatively longer cultivation duration and lower ethanol yield per unit biomass. Ethanol yield is most favourable at site B (Kano) where SSV2 cultivar showed ethanol yield of 8381 L/ha, KSV3 showed 9420 L/ha and KSV8 showed 7004 L/ha respectively. Cultivar type and cultivation location may improve ethanol yields by over 25%. For example, both SSV2 and KSV8 sorghum crops produce more biomass under warmer and drier climatic conditions. Mild acid pre-treatment of sorghum bagasse at moderate temperatures followed by detoxification appeared to be a cost-effective platform for bioconversion of whole sorghum crop to ethanol. Further improvements in ethanol yield per hectare are envisaged through application of enhanced fermentation techniques such as very high gravity fermentation (VHG), immobilised yeast fermentation techniques among others.

CHAPTER FIVE

Analysis of sorghum bagasse benefits as fermentation feedstock for bioethanol production

5.1 Introduction

Sorghum is an important cereal in Nigeria. It is a high biomass yielding crop that produces 65-120 t/ha of fresh lignocellulosic biomass (Billa, *et. al.*, 1997). On harvest, typical residual lignocellulosic biomass generated includes crushed stalks (after juice extraction), crop head materials and leaves. These total residual lignocellulose biomass are herein referred to bagasse for the purpose of this study. In spite of the estimated, 2-3 million metric tons of dry sorghum bagasse generated annually in Nigeria, less than 30% of it are utilised as livestock feed or domestic cooking fuel. Bulk of the remains are either left in the field and/or burnt (Yevich and Logan, 2003; PROMISO, 2008; Makinde *et al.,* 2011). Consequently, in spite of investigations into the potential for bioconversion of SSV2, KSV8 and KSV3 sorghum cultivars grains, stalk juices and bagasse to ethanol as described in the previous chapters of this thesis, due to food security concerns relating to use of grains and stalk juice for bioethanol production, the bagasse fraction was considered as the most favourable feedstock for bioethanol production in Nigeria. The bagasse does not directly conflict with national food security supplies compared to stalk juice or even degraded grains, whose adequate supply may not be guaranteed when post-harvest grain storage facilities are appropriately developed around the country (Abila, 2010). In Chapter 4, results showed that SSV2, KSV8 and KSV3 sorghum cultivars bagasse exhibited varied fermentation performances which may be

attributed to the differences in cultivar type and the cultivation location (summarised in Table 5.1). Therefore, it was considered expedient to further investigate which of these sorghum feedstocks had the most favourable aggregate benefits in terms of selected economic, social and environmental attributes. Multi-criteria analytical methodology was employed as a tool to carry out this analysis using a simplified approach. The steps according to Watson and Buede (1989) involve:

1. Establishing the decision context i.e. the principle statement.
2. Identifying the criteria to be used.
3. Involving some experts to assign ranking and rating to the selected criteria.
4. Scoring and weighting of each criterion.
5. Combining the weights and scores for each criterion to derive corresponding aggregate benefits.
6. Conducting sensitivity analysis on the aggregate benefit results.

Table 5.1 Summary of sorghum bagasse composition and fermentation yields per hectare of farmland

	Kano			Kaduna	
Parameters	**SSV2**	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Sorghum bagasse yield (t)	28.6	32.72	36.83	24.31	30.49
Sugar yield, from detoxified bagasse hydrolysates (t)	17.64	14.53	22.42	14.07	12.47
Total energy input[a] (MJ)	14,008	11,839	15,815	9,072	11,083
Ethanol energy output[b] (MJ)	176,755	147,714	198,668	113,338	138,308
Energy ratio (output[b]/input[a])	12.62	12.48	12.56	12.49	12.48
Observed ethanol yield (L)	8,381	7,004	9,420	5,374	6,558
Theoretical ethanol yield (L)	11,425	9,411	14,520	9,113	8,076
Fermentation efficiency (%)	73.4	74.4	64.9	59.0	81.2
GHG, from sorghum cultivation & bagasse burning ($kgCO_2$ eq.)[C]	105.877	121.130	136.345	89.996	112.874
GHG, from sorghum cultivation & ethanol combustion ($kgCO_2$ eq.)[d]	8331	6973	9369	5349	6528
Observed CO_2 from fermentation (kL)	106.35	77.97	114.84	61.90	69.60

[a]Total energy input includes sorghum crop cultivation/harvesting energy requirement at 15.05 MJ/t bagasse and energy requirement for sorghum bagasse pre-treatment, hydrolysis and fermentation to ethanol at 1.62MJ/L ethanol produced (Koonin, 2007; Groode and Heywood, 2007; Schmer *et al.,* 2008).

[b]Ethanol energy output at ethanol lower heating value (LHV) of 21090 KJ/kg ethanol (Walker, 2010).

[c]Estimated cumulative GHG emission (as $kgCO_2$ equivalent) from sorghum crop cultivation/harvesting at 1.175 $kgCO_2$ eq./t and sorghum bagasse burning at 2.527 $kgCO_2$eq./t bagasse (Nasidi *et al.,* 2010).

[d]Estimated cumulative GHG emission (as $kgCO_2$ equivalent) from sorghum crop cultivation/harvesting at 1.175 $kgCO_2$ eq./t and ethanol combustion at 0.99 $kgCO_2$ eq./L ethanol (Nasidi *et al*., 2010).

5.2. Multi-criteria analysis (MCA) method

Almost all our everyday decisions are based on a multi-criteria analysis approach (DCLG, 2009). Multi-criteria analysis (MCA) may be defined as a decision-making tool that was developed for solving complex multi-dimensional problems taking into consideration the qualitative and/or quantitative objectives of the problem in the decision-making process (Godwin and Wright, 1999). In this study, multi-criteria analysis was undertaken to make a benefit comparative assessment between SSV2, KSV8 and KSV3 sorghum bagasse cultivated from Kano and Kaduna (i.e. sites B and Z) as fermentation substrates in terms of social, economic and environmental attributes. For simplicity, Simple Multi-Attribute Rating Technique (SMART) was employed (Wang *et al.,* 2009), because the method does not involve mathematical "black box" complexities, it is fairly easy and fast to implement. However, due to its simplicity, certain detailed aspects of the decision objectives may not be captured in the process. Nevertheless, the method has been widely adjudged to be robust and suffices for fast decision making purposes (Watson and Buede, 1989).

The objective of this study was to identify the crop residue from SSV2, KSV8 and KSV3 sorghum cultivar that will be the most favourable feedstock source for bioethanol production in Nigeria taking into consideration some aspects of environmental, social and economic attributes respectively. Belton and Stewart (2002) have previously reported a simple systematic approach for undertaking Multi-Criteria Analysis using the SMART concept that involves six sequential stages. The concept was adopted in this study as enumerated below.

Stage 1- Establishing the decision principle: in this study, SSV2, KSV8 and KSV3 sorghum bagasse cultivated in Kano and Kaduna were bio-converted to fuel alcohol with carbon dioxide as co-product. Results of the study (Chapter four) suggested sorghum cultivar type and cultivation location influenced the fermentation performance of the bagasse substrates. Therefore, it was desired to identify the sorghum substrate that has the highest aggregate benefit score as a fermentation feedstock source taking into consideration some social, environmental and economic attributes in the decision taking.

Stage 2- Identification of attributes and criteria: the criteria identified in this study may be classified under Environmental, Social and Economic attributes respectively (Fig. 5.1).

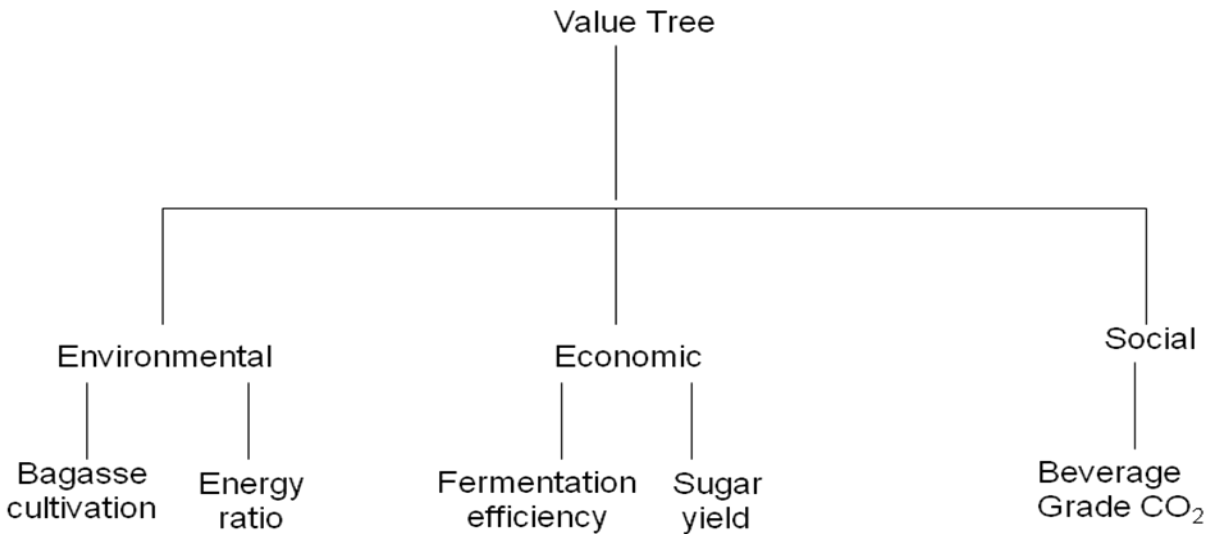


Fig.5.1 Value tree chart. Represention of social, economical and environmental criterion and their respective indicators. Source: Goodwin and Wright (2001)

In Fig. 5.1, sorghum cultivation/harvesting and the energy output/input ratio are considered environmental related attributes while improved fermentation

performance and fermentable sugar yields were associated with economic attributes bearing in mind these are considered in this context to be capital investment related costs. Finally, the carbon dioxide (CO_2) produced during fermentation was considered a social attribute because it is envisioned the produced carbon dioxide would be further refined and utilised in the food and beverage industry.

Stage 3- Identifying stakeholders and data collection: MCA outcome is heavily dependent on input from experts and stakeholders. However, due to time and resource constrains in this study, a limited number of experts i.e. eleven respondents were engaged for input into this study. The respondents were all University lecturers in Nigeria with different academic and professional background. The respondents were asked to judge the importance of each criterion (Table 5.2) relative to the Principle statement, particularly in the context of environmental, economic and social impact on cultivation and utilisation of sorghum bagasse as bioethanol feedstock.

Principle statement: *Sorghum crop residue (bagasse) is a favourable feedstock source for bioethanol production in Nigeria.*

Table 5.2 Criteria attributes to decision making

Criteria
1. Selecting appropriate sorghum cultivar and the cultivation location improves ***bagasse yield*** by 10-15%
2. High output/input ***energy ratio*** will improve environmental well being.
3. Improved ***fermentation efficiency*** favours higher ethanol.
4. Efficient bagasse pre-treatment improves fermentable ***sugar yield*** per unit bagasse substrates.
5. With improved fermentation efficiency, comes corresponding increase in ***CO_2 gas production*** from fermenting broth; CO_2 gas is to be utilised in beverage production/packaging.

Stage 4- Ranking and Rating: to arrive at a preferred option with maximum aggregate benefits (Mabin and Beattie, 2006), respondents were required to rank and rate the importance of each criterion relative to attributes of the decision to be considered in this study. The ranking methodology involves assigning each criterion a rank that reflects its perceived degree of importance relative to the decision being made by the respondent, the most important criterion being number 5 and the weakly important criterion being number 1, the rating scoring is similar to ranking, except that the rating involves assigning corresponding 'grades' between 0 and 100 to each ranked criterion (Table 5.3).

Table 5.3 Ranking and Rating scores for criterion

Ranking	**Rating/grades**
Most important = 5	80-100
More important = 4	60-79
Moderately important = 3	40-59
Less important = 2	20-39
Weakly important = 1	0-19

Hence, respondents were required to rank criterion in order of importance to them on a scale of 5 to 1 and to further assign grades from 0 to 100 to each of the ranked criterion. Table 5.4 shows the rankings and ratings of criteria according to respondent's responses while Table 5.5 shows summary results for the normalised rankings and ratings.

Table 5.4 The Rankings and Ratings of criteria by respondents

	Respondent 1		Respondent 2		Respondent 3		Respondent 4		Respondent 5		Respondent 6	
Criteria	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**
Energy ratio	5	85	4	75	3	45	5	85	4	65	4	75
Observed CO_2	2	30	3	50	5	90	3	50	4	60	5	85
Sugar yield	4	60	1	15	3	50	3	40	3	50	3	45
Ferm. Eff.	4	60	5	80	5	85	5	80	5	80	4	70
Bagasse yield	3	40	2	30	1	10	3	50	2	20	3	50

..... Continuation of Table 5.4

	Respondent 7		Respondent 8		Respondent 9		Respondent 10		Respondent 11			
Criteria	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Ranking**	**Rating**	**Sum of Ranking**	**Sum of Rating**
Energy ratio	4	70	3	50	4	65	5	90	3	50	44	755
Observed CO_2	4	65	5	85	5	90	4	60	4	70	44	735
Sugar yield	5	80	4	75	3	40	3	50	2	30	34	535
Ferm. Eff.	5	90	5	80	4	65	4	65	5	95	51	850
Bagasse yield	4	60	4	70	1	15	2	35	3	55	28	435
Cumulative											**201**	**3310**

Table 5.5 Normalised criteria Rankings and Ratings

Criteria	Sum of Ranking	Sum of Rating	*Relative Ranking wt.	**Relative Rating wt.	***Combined mean wt.
Energy ratio (output/input)	44	755	22	23	23
Observed CO_2, fermentation	44	735	22	22	22
Sorghum bagasse hydrolysate sugar yield	34	535	17	16	16
Fermentation efficiency	51	850	25	26	25
Sorghum bagasse yield (dry basis)	28	435	14	13	14
Cumulative	201	3310	100	100	100

*The relative Ranking weighting is the ratio of sum of each criteria to the cumulative Ranking × 100 (CIFOR, 1999) e.g. (44/201)*100 = 22.
**The relative Rating weighting is the ratio of sum of each criteria to the cumulative Rating × 100 (CIFOR, 1999) e.g. (265/1100)*100 = 24.
***The combined mean weighting is the average of the relative Ranking and Rating weighting (CIFOR, 1999) e.g. (22 + 24)/2 = 23.

Table 5.6 Evaluating the score for criterion

	Kano						Kaduna				
	SSV2		KSV8		KSV3		SSV2		KSV8		Sum of
	[a]Values	[b]Score	[a]Values	[b]Score	[a]Values	[b]Score	Values	Score	Values	Score	values
Energy ratio (out/in)	12.62	20.15	12.48	19.93	12.56	20.05	12.49	19.94	12.48	19.93	62.63
Ferment. efficiency	73.4	20.80	74.4	21.09	64.9	18.39	59.0	16.72	81.2	23.00	352.92
Observed CO_2	106.35	24.70	77.97	18.11	114.84	26.67	61.90	14.37	69.60	16.15	430.66
Bagasse yield	28.6	18.70	32.72	21.39	36.83	24.08	24.31	15.89	30.49	19.94	152.95
Sugar yield	17.64	21.74	14.53	17.91	22.42	27.65	14.07	17.34	12.47	15.36	81.13

[a]Values are results adopted from Table 5.1.
[b]Score are the normalised values e.g. (energy ratio)/(sum of values): (12.62/62.63)*100 = 20.15.

Table 5.7 Rationalised criteria scores and derived aggregate benefits

		Kano						Kaduna			
		SSV2		KSV8		KSV3		SSV2		KSV8	
Criteria	[a]**CW**	**S[b]**	**[c](CW * S)**	**S[b]**	**(CW * S)**	**S[b]**	**(CW * S)**	**S[b]**	**(CW * S)**	**S[b]**	**(CW * S)**
Energy ratio	23	20.15	463.45	19.93	458.39	20.05	461.15	19.94	458.62	19.93	458.39
Ferm. Efficien.	25	20.80	520.00	21.09	527.25	18.39	459.75	16.72	418.00	23.00	575.00
Observed CO_2	22	24.70	543.40	18.11	398.42	26.67	586.74	14.37	316.14	16.15	355.30
Bagasse yield	14	18.70	261.80	21.39	299.46	24.08	337.12	15.89	222.46	19.94	279.16
Sugar yield	16	21.74	347.84	17.91	286.56	27.65	442.40	17.34	277.44	15.36	245.76
Total			2136.49		1970.08		2287.16		1692.66		1913.61
Aggregate benefits			**21.37**		**19.70**		**22.87**		**16.93**		**19.14**

[a]CW refers to combined weighted ranking and rating (see Table 5.5).
[b]S represents normalised result values (see Table 5.6).
[c](CW * S) refers to product of combined criteria weighting and score e.g. 23 × 20.15 = 463.45.

Stage 5- Assigning normalised scores to criteria: for mathematical simplicity, the empirical data of the criteria obtained from previous section (chapter four), were converted to percentage ratio basis before being employed in the evaluation (Table 5.6). The aggregate benefits for each bagasse substrates are derived on a percentage basis (Table 5.7).

Stage 6- Sensitivity analysis and Decision making: sensitivity analysis, aimed to investigate degree of impact of criteria ranking and the rating scores on the aggregate benefits for the bagasse substrates. All the combined weighted scores were assigned weights of 100 and the resultant aggregate benefits are shown in Table 5.8.

Key highlights of the survey results indicated that the respondents placed high importance on selection of fermentation substrates in terms of their fermentation efficiency performance and their input/output energy value (Table 5.5). Analysis of the respondent's rankings in terms of empirical data generated in this study indicated SSV2 and KSV3 substrates in Kano has the most favourable aggregate benefits as fermentation substrates (Table 5.7). While SSV2 has the benefit of efficient fermentation performance and input/output energy value, the KSV3 has benefit of high biomass yield, which led to higher fermentable sugar yield (5.7). These results were in agreement with result of sensitivity analysis summerised in Table 5.9. Details of the relating to these findings are provided in the result and discussion section of this chapter.

Table 5.8 Sensitivity test for normalised criteria scores and derived aggregate benefits

		Kano						Kaduna			
		SSV2		KSV8		KSV3		SSV2		KSV8	
Criteria	**[a]CW**	**[b]S**	**[c](CW * S)**	**[b]S**	**(CW * S)**	**[b]S**	**(CW * S)**	**[b]S**	**(CW * S)**	**[b]S**	**(CW * S)**
Energy ratio	100	20.15	2015	19.93	1993	20.05	2005	19.94	1994	19.93	1993
Ferm. Efficien.	100	20.80	2080	21.09	2109	18.39	1839	16.72	1672	23.00	2300
Observed CO_2	100	24.70	2470	18.11	1811	26.67	2667	14.37	1437	16.15	1615
Bagasse yield	100	18.70	1870	21.39	2239	24.08	2408	15.89	1589	19.94	1994
Sugar yield	100	21.74	2174	17.91	1791	27.65	2765	17.34	1734	15.36	1536
Total			10609		9943		11684		8426		9438
Aggregate benefits			**106.09**		**99.43**		**116.84**		**84.26**		**94.38**

[a]CW refers to combined weighted ranking and rating (see Table 5.5).
[b]S represents normalised result values (see Table 5.6).
[c](CW * S) refers to product of combined criteria weighting and score e.g. 23 × 20.15 = 463.45.

Table 5.9 Sensitivity test for actual criteria scores and derived aggregate benefits

		Kano						Kaduna			
		SSV2		KSV8		KSV3		SSV2		KSV8	
Criteria	**[a]CW**	**[b]R**	**[c](CW * R)**	**[b]R**	**(CW * R)**	**[b]R**	**(CW * R)**	**[b]R**	**(CW * R)**	**[b]R**	**(CW * R)**
Energy ratio	100	12.62	1260	12.48	1248	12.56	1256	12.49	1249	12.48	1248
Ferm. Efficien.	100	73.40	7340	74.40	7440	64.90	6490	59.00	5900	81.20	8120
Observed CO_2	100	106.35	10635	77.97	7797	114.84	11484	61.90	6190	69.60	6960
Bagasse yield	100	28.60	2860	32.72	3272	36.83	3683	24.31	2431	30.49	3049
Sugar yield	100	17.64	1764	14.53	1453	22.42	2242	14.07	1407	12.47	1247
Total			23859		21210		25155		17177		20624
Aggregate benefits			**238.59**		**212.10**		**251.55**		**171.77**		**206.24**

[a]CW refers to combined weighted ranking and rating (see Table 5.5).
[b]R represents actual results (from Table 5.1).
[c](CW * R) refers to product of combined criteria weighting and actual results e.g. 23 × 20.15 = 463.45.

5.3 Discussion and conclusion

Fermentation efficiency followed by estimated total energy output-input ratio and carbon dioxide production parameters received the most favourable ranking and rating, respectively, by the respondents (Table 5.5). These results suggested that while the desire for higher ethanol yields through improved fermentation process is most important, concerns for energy use efficiency in the production process should equally be given diligent consideration and the economic gains associated with the production process should also be maximised. However, efficient bagasse pre-treatment methods leading to release of higher fermentable sugar yields received the least combined rating by respondents; this may be attributed to the fact that respondents arguably considered feedstock supply not to sufficiently warrant concern on the level of utilisation.

Furthermore, Table 5.7 showed that sorghum crop cultivation location is an important factor to consider when cultivating sorghum crop destined for lignocellulosic ethanol production, in particular when contemplating to establish integrated production facilities where carbon dioxide from fermentation process is considered as a co-product of the fermentation process i.e. in addition to the bioethanol being produced. Kano (site B) with warmer and drier climatic conditions was observed to be more favourable than Kaduna (site Z) with a relatively colder and wetter climate. Hence, the KSV3 bagasse substrate had the most favourable total aggregate benefit closely followed by SSV2 substrates, in Kano. However, the KSV8 bagasse substrate aggregate benefit does not appear to be significantly affected by cultivation location, though Kano appeared marginally more favourable. It is expedient to mention that among the weaknesses that may be observed with the multi-criteria analysis done here include:

1- The response from a total of eleven expert respondents consulted in this study may be arguebly considered inadequate for reliable decision taking. Their response alone may not be sufficient for chosing the most favourable feedstock for bioethanol production amongst the five studied in this thesis. Therefore, wider respondents groups that include stakeholders such as farmers, environmentalists and industrialists should be considered for inclusion. This is achievable through organising focus group meetings or fora where an overview of the whole bioethanol production process will be presented.

2- Further Multi-Criteria Optimisation will be required to determine the optimum crop cultivation management and bioethanol production process. This would guide on how to strike a balance between economic, social and environmental needs. This is to ensure reasonable sustainability criteria in the utilisation of sorghum bagasse.

3- Due to time constraints, limited criteria were selected in this study. More criteria such as GHG emissions, crop cultivation costs should be included in the analysis for broader decision taking.

4- This analysis aimed to rationalize simple approaches in the ‘complex problem’ of selecting favourable sorghum feedstock for bioethanol production in Nigeria. SSV2, KSV8 and KSV3 sorghum cultivated in Kano and Kaduna are the feedstock source. This is taking into cognisance of both objectives and subjective data of environmental, economic and social criteria respectively. However, the decision outcome may be argued as being too subjective and unreliable, but may suffice within scope limit of this work.

Finally, our findings indicated that of the three crops studied in this thesis, the SSV2 has highest benefit of shorter cultivation duration, most favourable ethanol and CO_2 yield over KSV3 and KSV8 substrates. Kano location appeared as the most favourable cultivation location as against Kaduna. Therefore, taking advantage of the realtively short crop season of the SSV2 and be cultivated twice or possibly thrice a year will be highly beneficial in terms of maximising aggregate biomass yield per annum thereby improving its energy output/input ratio with regards to fermentation.

CHAPTER SIX

Concluding discussion

Thesis snapshot

This thesis investigated the potential of sorghum as a feedstock source for bioethanol production in Nigeria. Sorghum is a two to three crop cycle per annum cereal. It exhibits high tolerance for varied environmental and climatic stresses. For example, sorghum thrives well under drought or water logged environment (Almodares and Hadi, 2009). While certain sorghum cultivars are essentially cultivated for their grains, others are cultivated either for their sweet stalk juice or high bagasse yield (Chopra, 2001). While the grains are utilised as staple food source or in brewing, the stalk juice may be used in syrup production. The green residues (bagasse) are partly used in forage production and fencing but mostly left in the field for burning (Nasidi *et al.,* 2010). Nigeria is the third largest sorghum producer worldwide, but less than 5% of sorghum produce in Nigeria has commercial application.

In this study, Nigerian local SSV2, KSV8 and KSV3 sorghum cultivars were grown in Kano and Kaduna (Nigeria). The crops were cultivated under rain-fed conditions and without chemical fertiliser application. While the climate in Kano is relatively warm and dry (33.5^{o}C, 340 mm precipitation), Kaduna is colder and wetter (26.5^{o}C, 600 mm precipitation). On harvest, our findings suggested Kano significantly ($p \leq 0.05$) favoured higher biomass yield while Kaduna favoured higher sugary stalk juice yield. The SSV2 showed most favourable raw juice yield of about 25500 L/ha at Kaduna

and about 25000 L/ha at Kano location. However, KSV8 showed corresponding raw juice yields of about 24500 L/ha and 23300 L/ha in Kaduna and Kano while KSV3 yielded about 22600 L/ha in Kano. With regard to bagasse yield, total dry bagasse yield in Kano was about 29 t/ha, 33 t/ha and 37 t/ha for SSV2, KSV8 and KSV3. The corresponding dry bagasse yield in Kaduna was 24 t/ha and 31 t/ha for SSV2 and KSV8 respectively.

The physico-chemical compositions of stalk juices and bagasse of SSV2 and KSV8 were observed to significantly vary between Kano and Kaduna. For example, while Kano crops favoured higher stalk juice starch accumulation, Kaduna crops favoured higher juice sugar accumulation. These observed characteristics were consistent with sorghum crop agronomy, being a C4 plant with efficient photosynthesis under warmer climate i.e. glucose being efficiently polymerised to starch (Almodares *et al.,* 2008). Furthermore, with regard to bagasse lignin contents, Kano crops bagasse showed higher lignin contents than the corresponding Kaduna bagasse, that may be related to the crop cell walls growing thicker to minimise moisture loss from stalk pith and inner structures under hot conditions (Yoshida *et al.*, 2008). However, proteins, amino acids and free amino nitrogen (FAN) of the juice and bagasse fractions did not show consistent variation trends between Kano and Kaduna. For example, while SSV2 showed significantly higher protein contents in Kano, KSV8 showed higher protein contents in Kaduna. These observations could be related to variations in the nitrogen, potassium and available phosphorus contents of the two locations as previously reported in scientific literature (Giginyu and Fagbiyide, 2009; Oduze and Kureh, 2009).

The experimental approach employed in this thesis for bioconversion of whole SSV2, KSV8 and KSV3 sorghum to ethanol involved using sugar, starch and lignocellulose platforms. For the sugar platform, stalk juices of SSV2, KSV8 and KSV3 were extracted by roller milling fresh stalks, the juice are clarified, filtered and sterilised. The juice sugar analysis by HPLC showed total fermentable sugar contents of 144 g/L, 66 g/L and 104 g/L for SSV2, KSV8 and KSV3 stalk juices in Kano and corresponding 162 g/L and 88 g/L sugars for SSV2 and KSV8 in Kaduna. Without further pH adjustment or exogenous nutrient supplementations, the juices were fermented by *Saccharomyces cerevisiae*. SSV2, with higher fermentable sugar, showed most favourable ethanol yields of 81 g/L in Kaduna and 65 g/L in Kano as determined by GC-MS. Furthermore, KSV8 showed 52 g/L ethanol in Kaduna and 36 g/L in Kano, respectively. However, KSV3 juice showed ethanol yield of 62 g/L. The observed ethanol yield of 81 g/L for SSV2 (Kaduna) compares favourably with 86 g/L ethanol yield reported by Zhao *et al*. (2012); for raw sorghum juice fermented by *S. cerevisiae* after supplementation with additional nutrients (urea, DAP, and $MgSO_4$). However, residual sucrose sugar observed in the fermented broth of SSV2 suggested nitrogenous compound is the limiting nutrient for its efficient fermentation. This indicated the possibility of improved ethanol yield from this substrate.

Thus, KSV3 and SSV2 (Kano) with observed higher FAN and amino acid contents were used to enrich the nitrogenous content of SSV2 (Kaduna) by blending in a ratio of 30:70 volumes i.e. 30 mL of either KSV3 or SSV2 (Kano) to 70 mL of SSV2 (Kaduna). After hydrolysis of the juice blends with exogenous amylase/protease enzymes supplementation, the substrates were fermented by *Saccharomyces cerevisiae* without additional exogenous nutrient supplementation. The SSV2 (Kaduna) juice supplemented with KSV3 (Kano) showed ethanol yields of 95 g/L

while SSV2 (Kano) juice supplementation yielded 92 g/L ethanol. These results represented improved ethanol yields of 15% and 12% over SSV2 (Kaduna) raw juice fermentation. The ethanol yield of 95 g/L for SSV2 (Kaduna) juice blended with KSV3 (Kano) compares favourably with 98 g/L reported by Ariyajaroenwong *et al.* (2012). Their results were based on re-concentration of sorghum stalk juice to 230 g/L total sugars and fermented by *S. cerevisiae*. Finally, the observed ethanol yields of 95 g/L and 92 g/L reported in this thesis are higher than the 86 g/L ethanol yields reported by Zhao *et al.* (2012) after supplementation of sorghum stalk juice with Urea/KH_2PO_4/$MgSO_4$ and fermented by *S. cerevisiae* yeast.

Finally, our findings suggested Kaduna favoured higher ethanol yields than Kano by up to 20% and 30% in terms of SSV2 and KSV8 raw juice substrates. SSV2 juice was found to be the most favourable juice substrate for fermentation in Kaduna. However, the SSV2 juice (Kaduna) ethanol yield may be further improved by up to 20% through supplementation with KSV3 raw juice (Kano). The significance of this finding indicated SSV2 is a suitable fermentation substrate that may be efficiently fermented without the need for additional commercial nitrogen source supplementation. This result is beneficial economically when contemplating to utilise SSV2 juice as fermentation substrate for commercial scale bioethanol production. However, further costing analysis may be required to highlight the possible overall cost benefits that can be derived.

Concerning the starch to ethanol platform for SSV2, KSV8 and KSV3 grains. The husked grains comprising of husks, awns and pubescence materials were each hammer milled and directly mashed with several combinations of commercially available enzymes supplements. The most favourable enzymes cocktail yielded

fermentable sugars of 61 g/100g flour, 70 g/100g flour and 69 g/100g flour for SSV2, KSV8 and KSV3, respectively. The total fermentable sugars analysed by HPLC in this context comprised glucose, maltose and xylose, of which glucose and maltose are predominantly liberated from starch hydrolysis while xylose is liberated from lignin degradation. The KSV3 and KSV8 substrates showed similar viscogram profiles along with high peak and final viscosity results (analysed by Rapid-Visco Analyser, RVA). Hence, the KSV3 and KSV8 with similar pasting profiles showed higher sugar yields while SSV2 with relatively lower peak and final viscosity showed lower sugar yields. Previous scientific literature suggested that starches with relatively high peak and final viscosities are likely to compose relatively high amylopectin which is more amenable to hydrolysis than amylose during hydrolysis (Agu *et al.,* 2006). Furthermore, SSV2, KSV8 and KSV3 mashes were observed to contain FAN levels above 150 mg/L, which was considered sufficient to support efficient fermentation process (Thomas and Ingledew, 1992).

Fermentations of these mash substrates with *Saccharomyces cerevisiae* without exogenous nutrient supplementation showed ethanol yields of 355 L/t, 421 L/t and 379 L/t flour for SSV2, KSV8 and KSV3 respectively. However, *Pichia stipitis* showed lower corresponding ethanol yields of 271 L/t and 272 L/t flour for the SSV2 and KSV8 mash substrates respectively. Corresponding fermentation CO_2 yields were observed by ANKOM^{RF} gas monitoring system. The total CO_2 evolved for SSV2, KSV8 and KSV3 mash fermentation with *Saccharomyces cerevisiae* were 81 kL/t, 94 kL/t and 89 kL/t flour, respectively. *Pichia stipitis* evolved a total of 49 kL/t and 59 kL/t corresponding CO_2 for SSV2 and KSV8 mash fermentations. The observed ethanol yields of 379 L/t and 421 L/t of KSV3 and KSV8 were similar to 380-390 L/t ethanol yields reported by Sheorain *et al.*, (2000) for de-husked and un-malted sorghum

grains mashed with commercial enzymes supplements and fermented by *S. cerevisiae*. It is pertinent to mention that other previous scientific literature has reported ethanol yields of 460-490 L/t for malted and pearled sorghum grains mashed with exogenous enzyme supplements (Agu *et al*., 2006; Ogbonna, 2011; Okolo *et al*., 2011; Serna-Saldívar *et al*., 2011; Aregbesola *et al.*, 2012). In this thesis, the fermentation of KSV8 and KSV3 un-malted and husked sorghum grains yielded favourable ethanol yields of over 390 L/t of crude flour. These results were obtained without supplementation of the fermentation worts with external nutrients. This finding is important because it suggested degraded or spoilt sorghum grains may be utilised as efficient feedstock source for bioethanol production with minimal commercial nutrients supplementation.

With regards to the lignocellulose to ethanol platform, oven dried SSV2, KSV8 and KSV3 bagasse were hammer milled. The crops harvested in Kano showed significantly higher observed starch and lignin contents relative to corresponding crops harvested in Kaduna. The milled bagasse samples were hydrolysed with dilute sulphuric acid at 75^{o}C for 3 h and followed by saccharification with a hydrolytic enzymes cocktail. Hydrolysates of SSV2, KSV8 and KSV3 from Kano yielded total fermentable sugars of 69 g/100g, 54 g/100g, 67 g/ 100g dry bagasse while total fermentable sugar of 66 g/100g and 50 g/100g dry bagasse were obtained for SSV2 and KSV8 hydrolysates in Kaduna. On fermentation of the hydrolysates with *Pachysolen tannophilus,* ethanol yields of 13 g/L, 9 g/L and 12 g/L was obtained for SSV2, KSV8 and KSV3 Kano while 11 g/L and 9 g/L ethanol was obtained for SSV2 and KSV8 in Kaduna. However, *Saccharomyces cerevisiae* yielded 12 g/L, 7 g/L and 12 g/L ethanol for SSV2, KSV8 and KSV3 hydrolysate (Kano) and 11 g/L and 8 g/L for SSV2 and KSV8 (Kaduna) hydrolysates respectively. Our observed 12-13 g/L

ethanol yields (SSV2, Kano) correspond to similar ethanol yields of 14 g/L reported by Ban *et al*., (2008); for sorghum bagasse pre-treated with phosphoric acid (80 g/L H_3PO_4) at 120^oC for 80 min and fermented by *P. tannophilus.* However, residual sugars and FAN were observed in the fermented broths.

The enzymatic hydrolysates were over-limed with anhydrous calcium hydroxide to precipitate out toxic organic acids which inhibit efficient fermentation process. This is followed by charcoal filtration to remove phenolics which are also inhibitory compounds generated from acid pre-treatment step. About 5% of the total fermentable sugars along with nitrogenous compounds were lost after successive over-liming and charcoal filtration of the hydrolysates. Fermentation of the final charcoal filtered hydrolysates showed notable improvement over the raw enzymatic hydrolysates. SSV2 followed by KSV3 (Kano) charcoal filtered hydrolysates showed ethanol yields of 23 g/L and 20 g/L on fermentation with *Pachysolen tannophilus* without exogenous nutrient supplementation. Furthermore, *Saccharomyces cerevisiae* yielded corresponding 21 g/L and 19 g/L for SSV2 and KSV3 (Kano). However, KSV8 charcoal filtered hydrolysates (Kano and Kaduna) as well as SSV2 (Kaduna) showed similar ethanol yields of about 17 g/L when fermented with either of *Pachysolen tannophilus* or *Saccharomyces cerevisiae*.

Our bagasse fermentation results indicated that Kano favoured higher ethanol yield relative to Kaduna. *Pachysolen tannophilus,* a xylose fermenting yeast, consistently appeared to be most efficient yeast for fermentation of lignocellulose hydrolysates than *Saccharomyces cerevisiae.* These ethanol yield results were corroborated by observed CO_2 evolution rates of the substrates fermentations. The results indicated SSV2 bagasse as the most favourable fermentation feedstock and its ethanol yield

potential may be improved by over 15% through de-toxification of its acidic hydrolysate. Our findings further suggested that sorghum bagasse may be possibly acid hydrolysed at moderate temperatures thereby reducing hydrolysis energy input. This is in addition to minimisation of rate of denaturing of proteins during hydrolysis; proteins are important source of yeast assimilable nitrogen sources.

Finally, we investigated the potentials of SSV2, KSV8 and KSV3 sorghums as feedstock sources for bioethanol production. The results indicated while SSV2 stalk juice and bagasse are most favourable fermentable sugar sources for bioethanol production, the KSV8 grain is the preferred starch source for ethanol production. However, taking into consideration some environmental, social and economic factors, KSV3 bagasse may be considered a sustainable feedstock for bioethanol production in Nigeria. The findings in this thesis are relevant to the aspirations of the Nigerian biofuels policy. The 2007 National biofuel policy of the Federal Republic of Nigeria mandated the use of E10 as alternative road transport fuel in Nigeria by 2020. However, Nigeria currently imports over 90% of its bioethanol requirement. Summary of the key results in this thesis were presented in Table 6.1.

Table 6.1 Summary of some key research findings**

Parameters	Kano			Kaduna	
	SSV2	**KSV8**	**KSV3**	**SSV2**	**KSV8**
Juice yield (L/ha)	25,024	23,304	22,570	25,596	24,536
Juice sugar yield (kg/ha)	3,603	1,534	2,346	4,134	2,158
Juice ethanol yield (L/ha)	2,070	1,075	1,768	2,614	1,619
Bagasse yield (t/ha)	28.60	32.72	36.83	24.31	30.49
Sugar yield; detoxified bagasse hydrolysates (t/ha)	17.64	14.53	22.42	14.07	12.47
Observed bagasse ethanol yield (L/ha)	8,381	7,004	9,420	5,374	6,558
Observed bagasse fermentation CO_2 yield (kL/ha)	106.35	77.97	114.84	61.90	69.60
Crude grains starch yield (g/100g flour)	65.64	69.87	73.42	NA*	NA*
Observed grains ethanol yield (L/t)	354.67	420.89	378.49	NA*	NA*
Observed grains fermentation CO_2 yield (kL/t)	80.70	93.90	88.40	NA*	NA*
Crop cultivation duration (weeks)	11	16	16	11	16

*NA = Not Applicable; only grains grown in Kano are used.

**Data are summarised from Tables 2.3, 2.5, 3.4, 3.12, 4.8 and 4.10.

Future work

While it may be tempting to conclude in this thesis that variation in climatic conditions influenced the favourable biomass yields of SSV2 and KSV8 sorghums in Kano, many other factors not investigated here needed to be considered before drawing such conclusions. Hence, the future direction of this work will include:

- ✓ The soil properties of Kano and Kaduna sites need to be investigated to determine the soil quality and investigate its effect on the composition of sorghum fermentation substrates biomass.

- ✓ More sorghum cultivars such as SK5921, SSV9 and KSV11 and two or more sites need to be considered for further investigation. This is to determine the role or effect of climatic condition of sorghum crops compositions. The study should ideally involve at least two crop seasons.

- ✓ Moderate chemical fertilizer application during crop cultivation is worth studying to investigate its effect on the biomass compositions.

- ✓ Furthermore, the effect of germinating the sorghum husked grains, followed by supplementation of the mashing liquor with appropriate amount of malted barley will be interesting to investigate.

- ✓ Determination of the fermentation inhibitory compounds concentrations in bagasse hydrolysates before and after fermentation will be interesting to investigate. This will give additional insight to the fermentation performance of each substrate.

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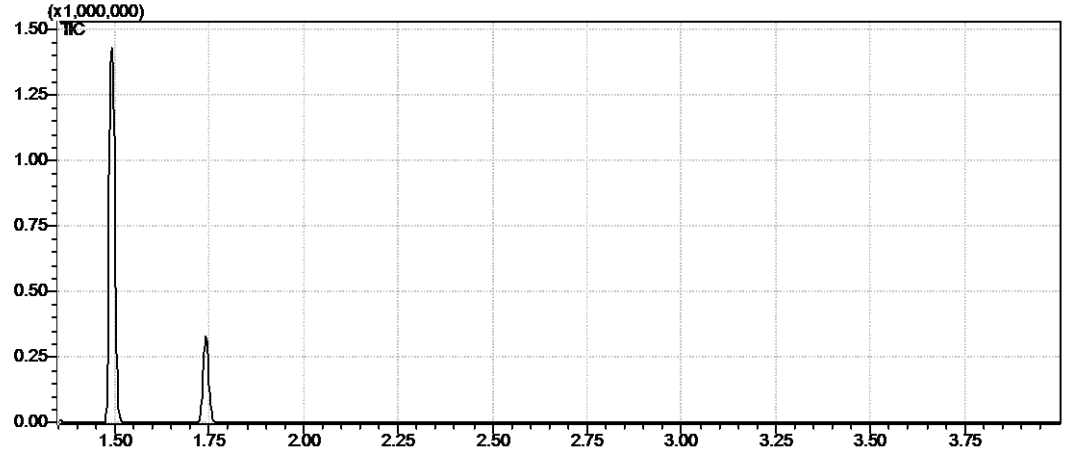
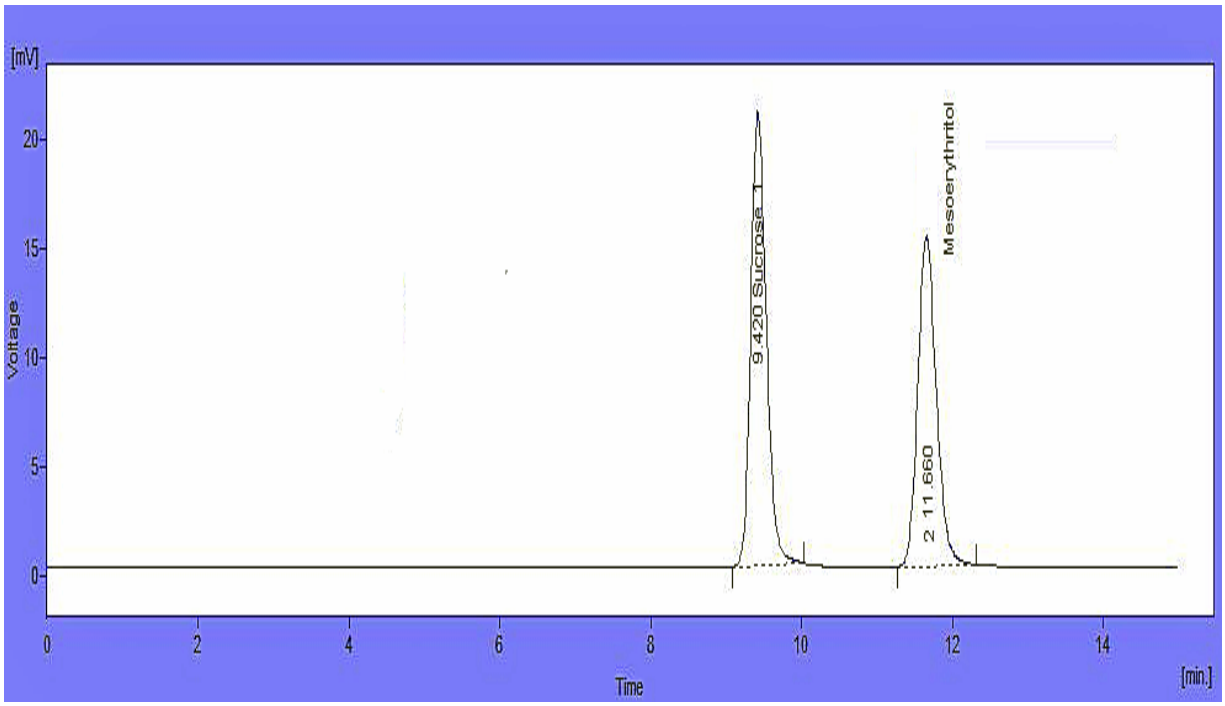
Appendices



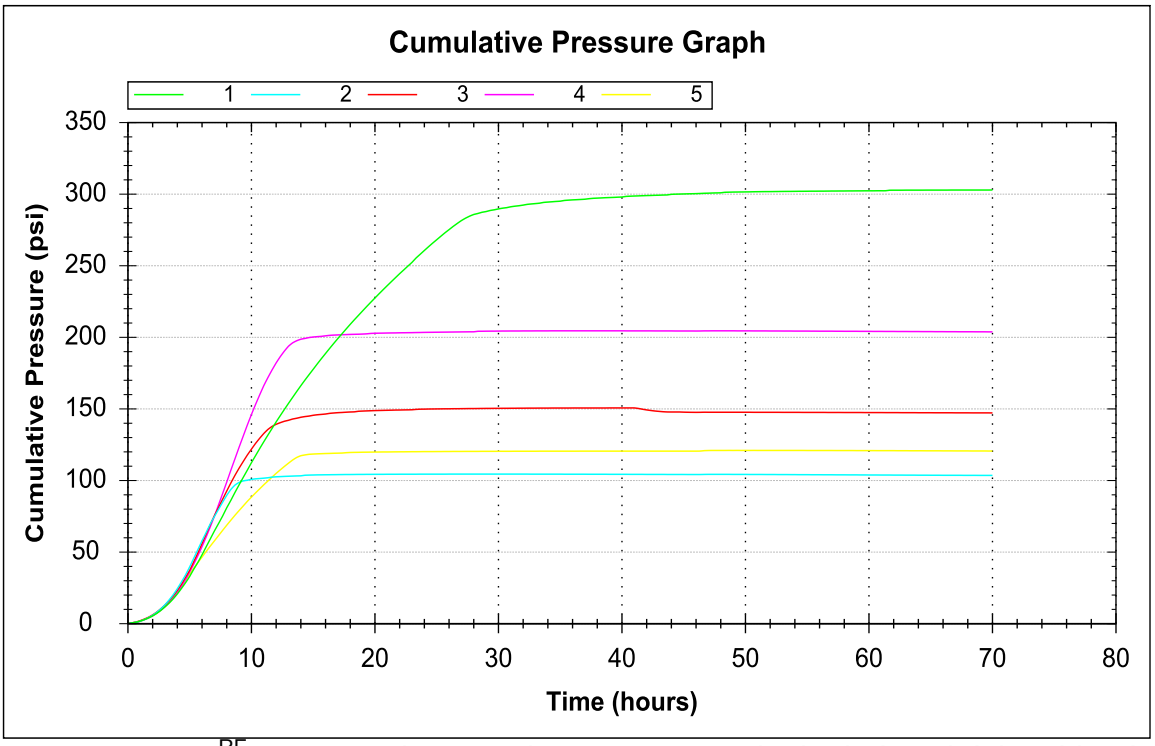
A. 1: GCMS-QP2010 ethanol chromatogram for SSV2Z juice broth: after 48 h of fermentation.

Corresponding quantitative ethanol yield for SSV2Z broth after 48 h fermentation

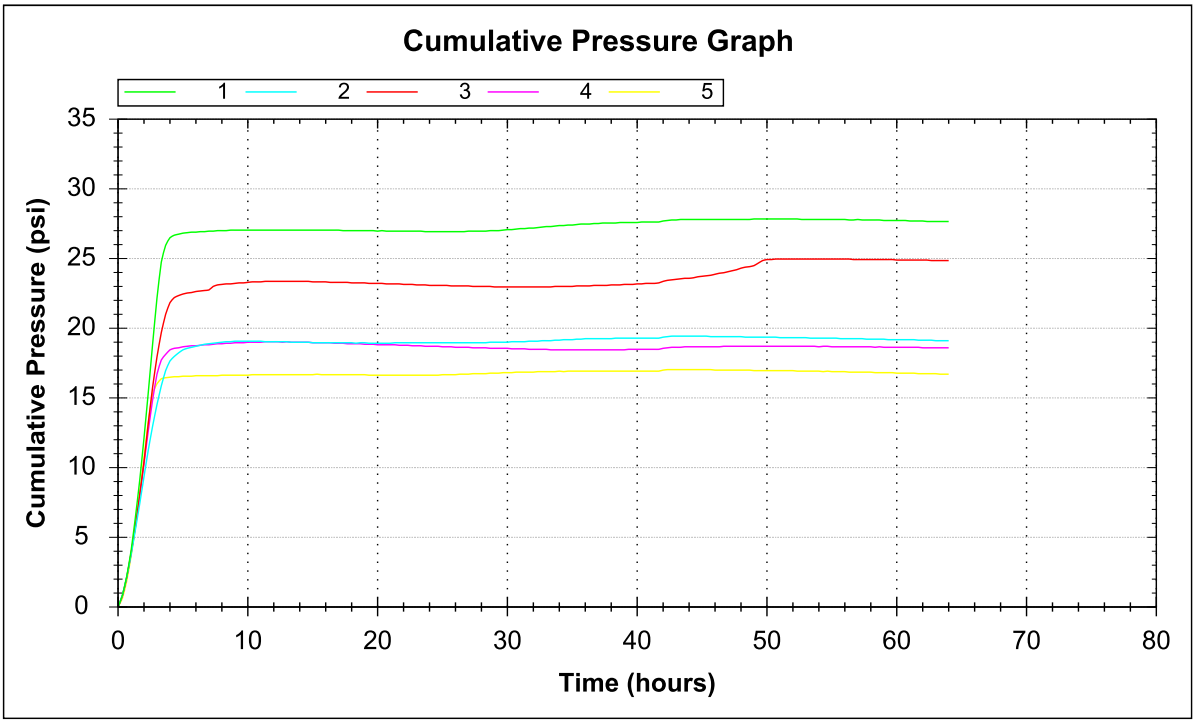
ID #	R. Time	m/z	Area	Height	Conc. (v/v)	Name
1	1.492	45.00	657215	671526	9.343%	Ethanol
2	1.740	42.00	44275	40209	1.000%	1- propanol



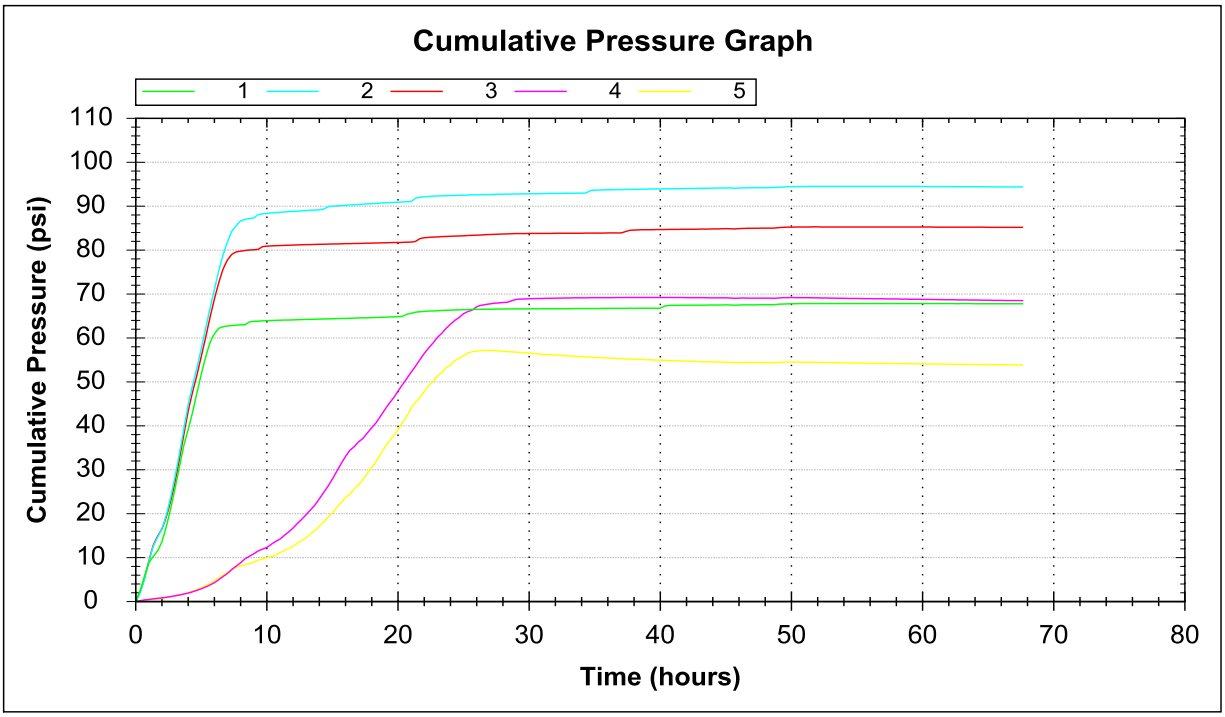
A. 2: HPLC Chromatogram for SSV2Z juice after 48 h fermentation. 1st peak from left represent residual sucrose and 2nd peak *Meso-erythritol* internal standard sugar (as control sample).



A. 3: ANKOM^{RF} fermentation graphs for enzymatic hydrolysed juice blends. Where; **1** = (SSV2Z + KSV3B), **2** = SSV2B, **3 =** SSV2Z, **4 =** (SSV2Z + SSV2B) & **5** = (SSV2B + KSV3B).



A. 4: ANKOM^{RF} fermentation graphs for charcoal filtered bagasse hydrolysates. Where; **1** = SSV2B, **2** = SSV2Z, **3** = KSV3B, **4** = KSV8B & **5** = KSV8Z.



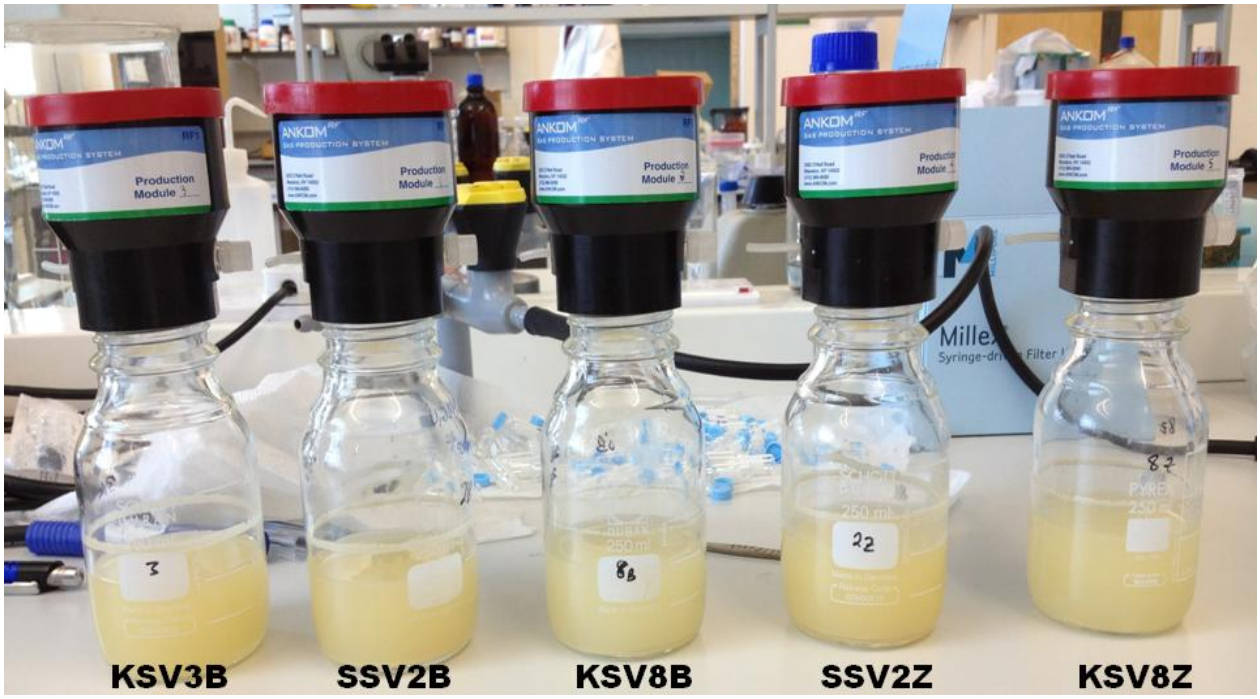
A. 5: Crude flour mash fermentation by CO_2 pressure monitoring using ANKOM^{RF}. Where; (**1** = SSV2, **2** = KSV8 & **3** = KSV3) are *S. cerevisiae* fermentations and (**4** = KSV8 & **5** = KSV3) are *P. stipitis* fermentations.

A. 6: Pre-matured SSV2 sorghum heads harvested in Kano and Kaduna. Crops were harvested 11 weeks after planting date. Kano (site B) grain appeared more matured than the corresponding Kaduna harvest (site Z). Note mold infections on grains.

A. 7: Freshly harvested SSV2, KSV3 and KSV8 sorghum stalks from Kano (site B)

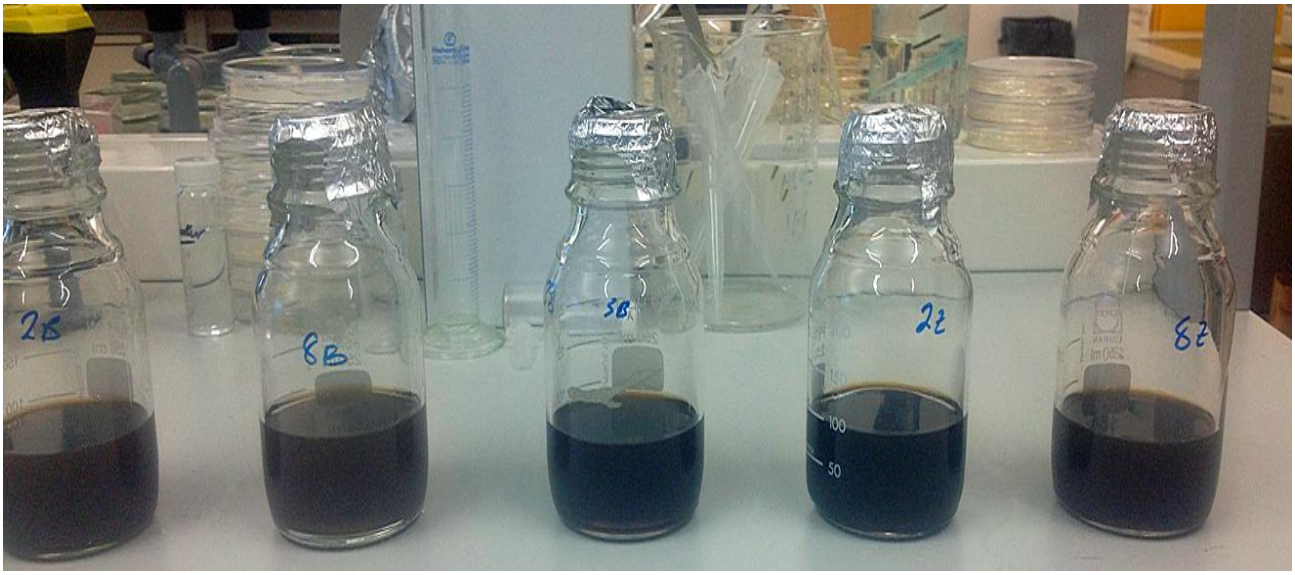
A. 8: Weighing of sun-dried sorghum residues.

A. 9: Hammer milled and oven-dried bagasse of SSV2, KSV8 and KSV3 sorghum samples from Kano (site B) and Kaduna (site Z).



A. 10: Charcoal filtered bagasse hydrolysates after 68 h fermentation with *P. tannophilus* in ANKOM^{RF} bottles. The bottle caps are ANKOM^{RF} modules.

A. 11: Sorghum stalk juice extraction by roller mill (Ohaus, Switzerland).

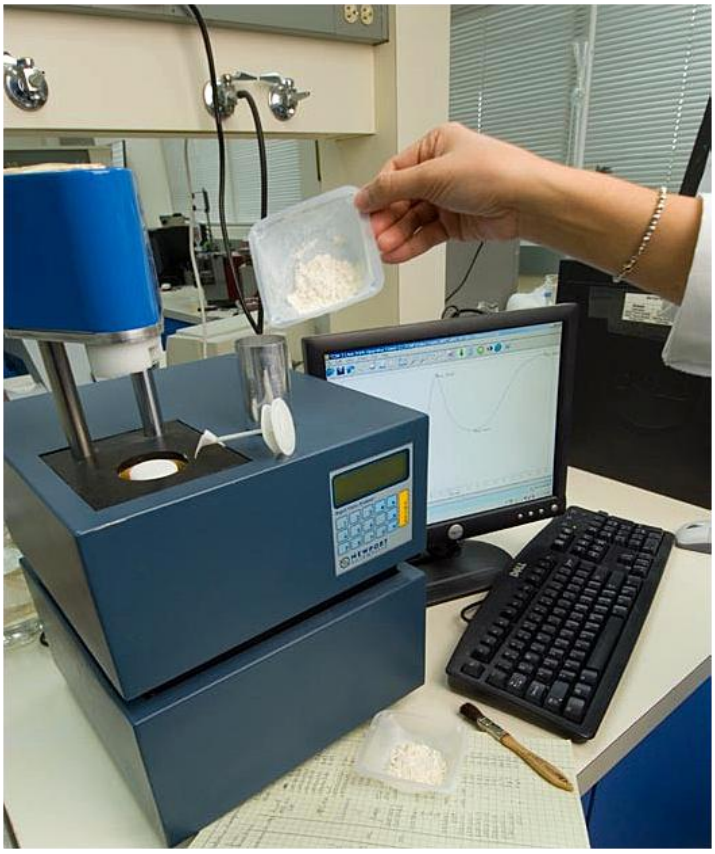


A. 12: SSV2, KSV8 and KSV3 sorghum raw stalk juice. Raw juices were filtered and prepared for fermentation with ANKOM^{RF}. Samples were extracted from Kano (site B) and Kaduna (site Z) harvesting locations. From left: SSV2B, KSV8B, KSV3B, SSV2Z and KSV8Z juice samples respectively.

A. 13: FermentationFlash (Funke-Gerber™, Berlin). The equipment used for alcohol concentration determination in this study.

A. 14: Shimadzu GCMS-QP2010 (Shimadzu Corporation, USA). Alternative equipment used for alcohol concentration determination in this study.

A. 15: HPLC autosampler (Spectra-physics, USA). Equipment used for sugar analysis in this study.



A. 16: RVA-4™ Rapid Visco Analyzer (Newport Scientific, Australia). Equipment used for stach pasting property analysis in this study.

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Fermentation of stalk juices from different Nigerian sorghum cultivars to ethanol

Abstract

For improved production of ethanol from sorghum stalk juice fermentation, cultivation location and cultivar type are important factors to consider. In the present study, SSV2 and KSV8 sorghum cultivars were cultivated in Kano and Kaduna states in Nigeria that exhibit notably different rain precipitation and diurnal temperatures. The crude stalk juices (without pre-treatment or nutrient supplementation) were extracted from these sorghum samples and fermented with a distiller's strain of the yeast, *Saccharomyces cerevisiae*. Sugar consumption and alcohol production were determined by HPLC and GC-MS, respectively. When it was grown in the Kaduna site, SSV2 was identified as the highest yielding sorghum cultivar from which we extracted the maximum levels of extractable sugars (161.50 g l^{-1}) that yielded favourable ethanol levels of 80.56 g l^{-1} following fermentation. Our findings show that relatively colder and wetter cultivation sites are preferred for sorghum stalk juice destined for bioethanol production.

Keywords

Sorghum cultivars • Varied climate condition • Juice composition • Fermentation performance • Bioethanol

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Abbreviations:

NIHORT: National Horticultural Research Institute, Nigeria.
PTDF: Petroleum Technology Development Fund, Nigeria.
Site **B**: Bagauda, Kano. Site **Z**: Zaria, Kaduna.

1. Introduction

As the world approached peak oil production era [1], there is increased uncertainty in predicting future prices of fossil fuels. Concerns on climate change due to continued use of fossil based fuels, has diverted the world attention towards developing renewable and sustainable transport fuels [2,3]. Fossil fuels are reported to account for over 80% of primary energy source globally, of which about 58% is expended as transport fuel [4]. In not too distant future biofuels comprising bioethanol, biobutanol, biodiesel, among others, are envisaged as likely alternatives to fossil fuels in transport sector, this is because of their renewability and sustainability over fossil fuels [5]. Bioethanol is strategically important as transport fuel of the future, because it is an environment friendly energy source which generates relatively acceptable quality exhaust gases leading to reduced GHG emissions [4,6]. Therefore, bioethanol as a plant-based liquid biofuel may be used in automobiles as additive or substitute to petroleum in transportation [7]. Nigeria is the 9th largest oil producing country in the world and largely depends on fossil based fuels as cheap energy source. This has constrained the desired growth in the renewable energy sector in the country [8,9]. However, the rapid depletion of global oil reserves and the spiralling cost of crude oil in global markets will necessitate the search for alternative and sustainable transport fuels in Nigeria. Bioethanol can be produced by bioconversion of plant based sugar-rich crops such as sorghum, sugarcane, cassava, and sugar beet.

Interestingly, sorghum (Sorghum *bicolor (L.) Moench*) is the 5th most important cereal globally and 2nd in Africa. It is a staple food source to over 500 million people and is cultivated in over 45 million hectares of farmland worldwide. Global sorghum production is estimated at over 60 million metric tons annually, where Nigeria is ranked among the top 3 largest sorghum producing countries in the world [10-12]. Sweet sorghum stalk juice contains variable amounts of sugars, proteins and starch depending on the cultivar type, crop harvesting time and cultivation location [13]. The typical sugars are predominantly glucose, sucrose and fructose, while maltose, dextrin, maltotriose and other oligosaccharides may be present in sorghum stalk juice in low concentrations [14]. The stalk may be directly chewed for its sweet juice as a snack, or the juice can be mechanically

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extracted and processed into sweet syrup or sugar cake (locally called “mazarkwaila” in Nigeria). High glucose-containing juices with minimal starch content are preferred for syrup production; as this avoids gelling and crystallization problems occurring during juice cooking or during long term storage of the finished syrup [15].

The Nigerian government through its “Biofuel policy statement” of 2007 aspires to achieve self sufficiency in bioethanol supply domestically by the year 2020. Sorghum, among other crops, has been designated as potential feedstock sources for Nigerian bioethanol [9]. Although the grains of some Nigerian local sorghum cultivars have been extensively studied for their potential use in malting and brewing, little or no attention has been given to the potential of these sorghum stalk juices for bioethanol production. Harvesting of sorghum grains before they reach physiological maturity makes them suitable for immediate use in brewing processes, while the stalk juice may be utilised as a fermentation substrate [16-19]. Nigerian SSV2 and KSV8 sorghums are cultivated because of their high grain quality and are regarded as being very tolerant to biotic and abiotic environmental stresses [10,20]. However, despite sorghum’s high adaptation to adverse climatic conditions, high productivity output remains constrained by poor soil quality, low and erratic rainfall and low agro-chemical inputs during cultivation. This is even more evident in developing countries where agro-chemicals and irrigation cultivation costs are often beyond the reach of peasant farmers [21,22]. Consequently, it is desirable to investigate the impact of these parameters on sorghum agronomic characteristics and productivity. For example, soil physical quality (such as hydraulic, pH, density, particle size and distribution among others) profoundly effects how best the soil can be managed for optimum crop yields [23]. In this study, the reported [24,25] morphological and physical properties of soils at sites B and Z were summarised in Table 1.

Previous research on improving sorghum juice fermentation has focused on sorghum stalk juice pre-treatment e.g. enhanced juice clarification and pH optimisation [26], supplementing juice substrates with commercially available yeast nutrients [27,28], enriching juice sugar levels with e.g. cane molasses for very high gravity (VHG) fermentation [29,30], immobilizing fermenting yeast cells e.g. on corncob, sorghum stalks or entrapment in sodium alginate gel beads among others [31-33]. Hence, the identification of highly fermentable sorghum juice producing cultivars that require lesser nutritional supplements is highly desirable, especially in the context of effective and manageable local bioresource utilisation, technological viability and economic sustainability. Limited attention has been given to the effect of climatic and environmental conditions on raw sorghum juice fermentation performance [26,31,34-36]. We therefore investigated the influence of environmental cultivation conditions (rainfall and temperature) on fermentable juice yield among locally grown Nigerian sorghum cultivars. We identified a cultivar (SSV2) that demonstrated superior potential for bioethanol production.

2. Methods

2.1 Sorghum cultivation and harvesting

Nigerian sorghum cultivar seedlings SSV2 and KSV8 were cleaned and treated with metalaxyl fungicide chemical (Apron Star™, Nigeria) and planted in Kano (site B) and Kaduna (site Z) in Northern Nigeria (Figure 1). The crops were cultivated under rain-fed conditions and only cow dung manure was applied. The SSV2 sorghums were harvested 11 weeks after planting when their grains were observed to reach soft-dough stage, while the KSV8 sorghums were harvested 16 weeks after planting when their grains also reached their soft-dough stage. The crops were harvested manually by cutting stalks above ground. One hundred stripped and cleaned stalks of SSV2 and KSV8 (randomly selected from each corner of fields) were roller-milled to extract the stalk juices (Ohaus®, Switzerland). The freshly extracted crude juices were clarified by gravity settling in holding tanks prior to filtration through polyester filter bags™ (SS Bolting Cloth, China) and pasteurization at 65°C for 2 h [15,18] using juice steriliser™ (Dacheng, China). Juice samples were stored at -20 °C until further analysis.

2.2 Compositional analysis of sorghum juice

Total starch contents of juices were determined using an enzyme assay with Megazyme™ K-TSTA kits according to AACC (American Association for Clinical Chemistry) standard method 76.13. Total free amino nitrogen (FAN) was determined by K-Large 02/11™ (yeast available nitrogen, YAN) and K-PANOPA 02/11™ (primary amino acid nitrogen, PAN) assay kits (Megazymes, Northern Ireland). Crude protein contents were

Table 1. Soils physical and morphological properties of crop cultivation location.

Parameters	Site B	Site Z
pH	5.0	5.2
Org. C (g kg^{-1})	0.38	3.3
Total N (g kg^{-1})	0.08	0.53
Avail. P (mg kg^{-1})	0.56	1.8
Exchangeable bases (C mol kg^{-1})		
Ca	0.27	1.80
Mg	0.08	0.36
Na	0.30	0.05
K	0.19	0.33
Exch. Acidity (Al^{3+} H^{+})	0.24	0.10
CEC	1.08	4.0
Soil physical properties (g kg^{-1})		
Sand	78	46
Silt	12	40
Clay	10	14

Source: [23-25].

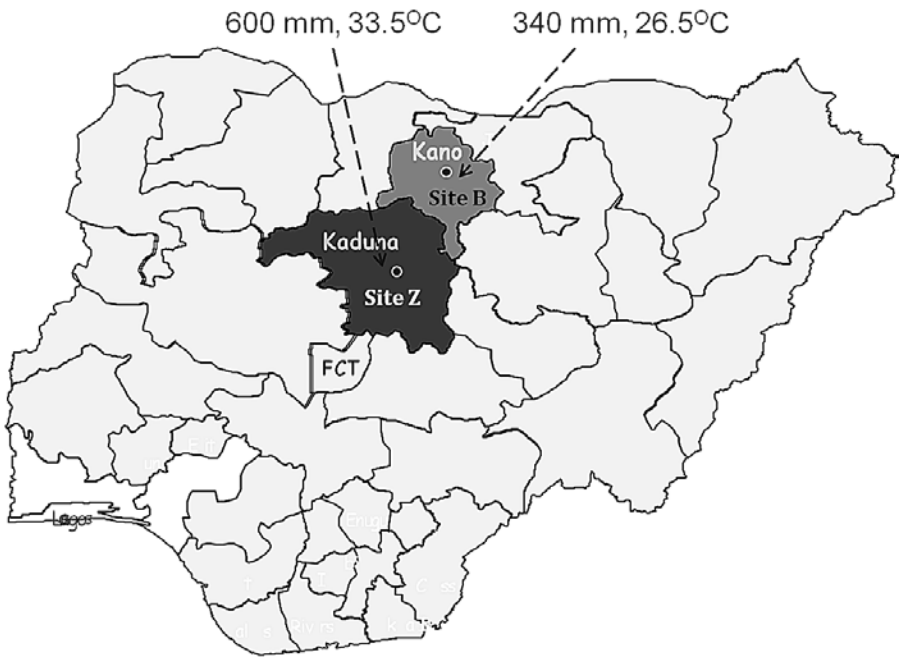

Figure 1. Map of Nigeria showing site locations B (coordinates: 11.33 °N, 8.23 °E) and Z (coordinates: 11.10 °N, 7.38 °E) as well as the climatic conditions where SSV2 and KSV8 sorghum cultivars were grown for the purpose of this study.

determined by Bradford's reagent (Sigma-Aldrich, UK) using recommended 3.1 ml protocol and the absorbance read with a Genesys® 10 s spectrophotometer (Thermo spectronic, USA). Total amino acid concentrations were determined courtesy of Heriot-Watt University Edinburgh by gradient elution method using HPLC equipment [37]. Briefly, fresh juice (2 ml) were filtered through 0.22 µm filters into HPLC-grade vials and placed in Gilson 231 autosampler with 40 I dilutor (Gilson, USA), the juice amino acids components were separated with a 150 mm × 4.6 mm phenosphere NEXT, 5u, C18 column™ (Phenomenex, UK) and detected by FP-1520 fluorescent detector (Jasco, USA), Gilson 715 data handling package was used to quantified amino acids. To determine the major fermentable sugars in juices (i.e. glucose, sucrose and fructose), 1 ml aliquots of sorghum juices (at 1:10 dilution) were filtered through 0.22 µm micro syringe filters into 2 ml vials containing 1ml *meso*-erythritol solution (internal standard sugar). The final solutions were vortexed and placed in an HPLC auto sampler (Spectra-physics, USA) and the sugars separated with a 300 mm × 7.8 mm REZEX RPM-monosaccharide pb+2 (8%) column™ (Phenomenex, USA) and quantified using HPLC software (CSW32 version v.1.4 chromatogram software from DataApex®, USA).

2.3 Yeast seed culture preparation

Yeast seed cultures were prepared by firstly inoculating two loop fulls of *Saccharomyces cerevisiae* (DCLM distillers' yeast strain, courtesy of Kerry Biosciences, Menstrie, Scotland) into 400 ml YEPD media comprising of 2.0% (w/v) yeast extract, 4.0% (w/v) bacteriological peptone, and 4.0% (w/v) glucose. Cultures were incubated at 32 °C with orbital shaking at 150 rpm for 20 h.

2.4 Fermentation and alcohol analysis

Frozen crude juices were thawed to room temperature and filtered through glass fibre filters (Millipore®, Sigma Aldrich). Appropriately washed cell pellets of *S. cerevisiae* yeast from the prepared YEPD culture were inoculated into 100 ml of raw juice at pitching rate of 10×10^6 cell ml^{-1}. Fermentation was then conducted at 32°C with orbital shaking at 130 rpm. Samples were withdrawn every 24 h for alcohol determination by gas chromatography using a GC-column (ZB-AAA; Phenomenex Inc, USA) on Agilent GC-MS model 6890GC (Agilent, Palo Alto, USA) equipped with MSD model 5975 Inert XL, PTV injector (Gerstel, Muehlheim, Germany).

2.5 Statistical analysis

Analysis of variance (ANOVA) by a GLM model using Minitab™ 16 statistical software (MINITAB©, USA) was used to test for significant differences in compositions of stalk juices of SSV2 and KSV8 sorghum cultivars grown in sites B and Z, respectively. Significant differences were tested at 95% confidence level and results with p values < 0.05 were considered significantly different.

3. Results and discussion

The mean recorded diurnal temperatures and rainfall for site locations in Kano (site B) were respectively 33.5 °C and 340 mm, and in Kaduna (site Z) were 26.5 °C and 600 mm (NIHORT, Nigeria). The sorghum crops were harvested before grains reached physiological maturity to avoid juice sugars getting converted to starch; allowing the sorghum grains to grow beyond soft-dough stage would result to conversion of the juice sugars predominantly to starch. The SSV2 sorghum cultivar showed

higher total stalk juice yield despite having less cultivation duration relative to KSV8 (Table 2). Whilst significant differences ($p < 0.05$) were observed in juice yields between SSV2 and KSV8 sorghums and accounted for about 22% in favour of SSV2, the observed significant differences ($p < 0.05$) in juice yields of SSV2 and KSV8 due to varied climatic conditions between sites B and Z appeared to account for over 10% differences in favour of site Z (Table 2). This suggests that sorghum cultivar selection is a very important factor to consider prior to site selection when cultivating sorghum crops for stalk juice production. In addition to quantitative stalk juice yields of sorghum cultivars from the present study [38,39], have considered the qualitative composition of juices as important criteria for sorghum cultivar selection destined for bioethanol production.

In the context of the effect of temperature and rainfall, the aggregate compositions of SSV2 and KSV8 stalk juices between sites B and Z shown in Table 2 were significantly different ($p < 0.05$). Site B favoured higher juice starch accumulation, whilst site Z favoured higher total contents of fermentable sugars (Table 2). These results were consistent with the C-4 agronomic characteristics of sorghum crops [13]. For example, the observed warmer and drier climatic condition of site B appeared to favour relatively higher biomass yield as observed from the bagasse yield shown in Table 2. Furthermore, higher protein content of juice was observed to correspond to higher FAN and amino acids concentrations in juice and vice versa as shown in Tables 2 and 3.This was suggested to be related to cultivation soil quality rather than rainfall or temperature variation effect as discussed by Roland and Gene [40].

The primary extracellular nitrogen sources for yeast biosynthetic activities during fermentation are individual amino acids, ammonium ions (NH_4^+) and small peptides. The NH_4^+ and small peptide molecules are principally derived from proteolytic catabolism of proteins and FAN available in juice. In addition to the extracellular free amino acids, *S. cerevisiae* cells are also able to utilised endogenous amino acids such as arginine as sole nitrogen sources [41,42]. Due to specificity and sensitivity limitations of Megazyme K-LARGE/K-PANOPA assay kits in detecting specific amino acids [43], individual amino acid concentrations in sorghum juices were determined by Gas chromatography-mass spectrophotometry. Data in Table 3 show SSV2 and KSV8 sorghum juices from sites B and Z contain all amino acids necessary for efficient yeast metabolism during fermentation. These amino acids were broadly classified into 3 groups based on *S. cerevisiae* cells orderly preferential uptake during fermentation [42]. Thus, group 1 amino acids were assimilated by yeast within 24 h of the onset of fermentation, and this mirrors the observed fast fermentation rates of SSV2Z juice (Figure 2) which has higher levels of group 1 amino acids along with higher FAN and glucose contents compared with SSV2B, KSV8B and KSV8Z juices (Tables 2 and 3). This nutritionally favourable combination of readily available nitrogen plus fermentable sugar in the SSV2Z juices explains the faster overall fermentation rates observed with this particular sorghum cultivar, as shown in Figure 2. The KSV8Z juice showed similar fast fermentation rates compared with SSV2Z juice in the first

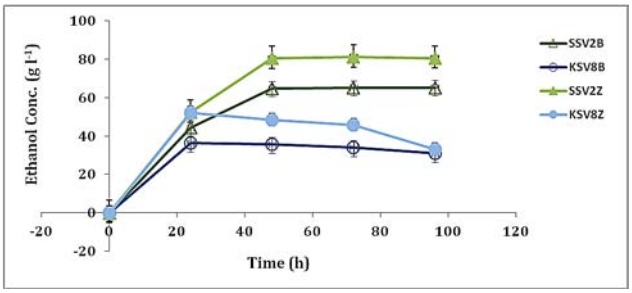

Figure 2. Ethanol concentration profile of sorghum stalk juice fermentation. Stalk juices fermentation performance for SSV2 and KSV8 sorghum cultivars fermented with S. cerevisiae at 32 °C and 120 rpm. Sorghum crops were grown in Kano (site B) and Kaduna (site Z), Nigeria. Std mean of duplicate experiments.

Table 2. Compositional analysis of SSV2 and KSV8 sorghum stalk juices.

cultivar	SSV2		KSV8	
	Site B	Site Z	Site B	Site Z
Dry bagasse (t ha^{-1})	35.60 ± 1.17^{a}	29.31 ± 1.92^{b}	39.72 ± 1.86^{c}	33.49 ± 1.24^{d}
Juice yield (l ha^{-1})	25024 ± 20.43^{a}	25596 ± 13.32^{b}	23304 ± 4.93^{c}	24536 ± 9.07^{d}
Total starch (g l^{-1})	0.97 ± 0.01^{a}	0.64 ± 0.01^{b}	0.51 ± 0.01^{c}	0.37 ± 0.01^{d}
Total protein (g l^{-1})	1.58 ± 0.01^{a}	1.82 ± 0.01^{bc}	1.08 ± 0.01^{d}	1.03 ± 0.01^{d}
Total FAN (mg L^{-1})	224 ± 1.14^{a}	325 ± 3.22^{b}	191 ± 1.43^{c}	134 ± 1.52^{d}
Sucrose (g l^{-1})	102.71 ± 3.76^{a}	113.93 ± 1.88^{b}	36.41 ± 2.11^{c}	55.67 ± 1.39^{d}
Glucose (g l^{-1})	27.58 ± 2.03^{a}	32.07 ± 1.14^{bc}	19.73 ± 0.83^{d}	21.76 ± 1.18^{d}
Fructose (g l^{-1})	13.69 ± 1.54^{ab}	15.50 ± 0.34^{ab}	9.67 ± 0.13^{cd}	10.52 ± 0.96^{cd}
Total sugars (g l^{-1})	143.99 ± 3.27^{a}	161.50 ± 3.36^{b}	65.81 ± 2.81^{c}	87.96 ± 3.53^{d}

The composition of crude stalk juices of SSV2 and KSV8 sorghum cultivars grown in Kano (site B) and Kaduna (site Z), Nigeria, under rain fed conditions and without application of chemical fertilizer. SSV2 and KSV8 were harvested 11 and 16 weeks after planting dates respectively. Results are Mean of triplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

Table 3. Amino acids profile of SSV2 and KSV8 sorghum stalk juices.

Cultivar	SSV2		KSV8	
Amino acids	Site B	Site Z	Site B	Site Z
Group 1: (μmole ml^{-1})				
aspartic acid	1.141 ± 0.011^{a}	0.730 ± 0.009^{b}	0.631 ± 0.007^{c}	0.530 ± 0.006^{d}
glutamic acid	0.444 ± 0.080^{ad}	0.538 ± 0.008^{bc}	0.403 ± 0.005^{ad}	0.462 ± 0.007^{ad}
asparagine acid	6.410 ± 0.120^{a}	10.580 ± 0.020^{b}	6.705 ± 0.070^{c}	3.885 ± 0.070^{d}
glutamine	3.145 ± 0.090^{a}	2.690 ± 0.080^{b}	1.545 ± 0.070^{c}	1.595 ± 0.070^{d}
serine	0.956 ± 0.007^{a}	0.750 ± 0.005^{b}	0.396 ± 0.007^{c}	0.345 ± 0.006^{d}
arginine	0.093 ± 0.004^{a}	0.082 ± 0.005^{b}	0.055 ± 0.006^{c}	0.030 ± 0.006^{d}
threonine	0.391 ± 0.007^{a}	0.293 ± 0.004^{b}	0.196 ± 0.007^{c}	0.125 ± 0.003^{d}
lysine	0.086 ± 0.005^{a}	0.076 ± 0.006^{b}	0.048 ± 0.003^{bc}	0.014 ± 0.003^{d}
Sub-Total	12.664 ± 0.234^{a}	15.738 ± 0.103^{b}	9.977 ± 0.025^{c}	6.985 ± 0.157^{d}
Group 2: (μmole ml^{-1})				
histidine	0.071 ± 0.007^{a}	0.033 ± 0.002^{bc}	0.061 ± 0.003^{a}	0.023 ± 0.004^{d}
methionine	0.027 ± 0.004^{a}	0.020 ± 0.004^{a}	0.013 ± 0.001^{bc}	0.007 ± 0.003^{d}
isoleucine	0.264 ± 0.006^{a}	0.293 ± 0.004^{b}	0.181 ± 0.006^{c}	0.133 ± 0.004^{d}
leucine	0.205 ± 0.008^{a}	0.275 ± 0.005^{b}	0.147 ± 0.005^{c}	0.105 ± 0.007^{d}
phenylalanine	0.192 ± 0.008^{a}	0.085 ± 0.004^{b}	0.086 ± 0.005^{b}	0.037 ± 0.007^{cd}
valine	0.644 ± 0.003^{a}	0.674 ± 0.008^{a}	0.354 ± 0.005^{bc}	0.241 ± 0.007^{d}
Sub-Total	1.402 ± 0.008^{a}	1.380 ± 0.003^{a}	0.841 ± 0.005^{bc}	0.544 ± 0.001^{d}
Other Groups: (μmole ml^{-1})				
glycine	0.109 ± 0.006^{a}	0.165 ± 0.006^{b}	0.064 ± 0.005^{cd}	0.076 ± 0.007^{cd}
alanine	0.967 ± 0.004^{a}	1.063 ± 0.008^{b}	0.434 ± 0.008^{c}	0.389 ± 0.005^{d}
proline	0.034 ± 0.008^{ad}	0.134 ± 0.008^{bc}	0.031 ± 0.005^{ad}	0.027 ± 0.005^{ad}
tryptophan	0.226 ± 0.006^{a}	0.070 ± 0.004^{b}	0.095 ± 0.004^{c}	0.042 ± 0.004^{d}
tyrosine	0.450 ± 0.005^{a}	0.181 ± 0.008^{bd}	0.231 ± 0.007^{c}	0.184 ± 0.010^{bd}
Sub-Total	1.786 ± 0.007^{ab}	1.613 ± 0.002^{ab}	0.853 ± 0.019^{cd}	0.717 ± 0.009^{cd}
Grand Total	15.852 ± 0.055^{a}	18.731 ± 0.046^{b}	11.671 ± 0.114^{c}	8.246 ± 0.231^{d}

Amino acid concentration (μmole ml^{-1}) in crude stalk juices of SSV2 and KSV8 sorghum cultivars grown in sites B and Z under rain fed condition and without chemical fertilizer application. Results are Mean of duplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

24 h, this was despite the former having lower glucose and group 1 amino acids contents than the latter (Tables 2 and 3). The KSV8Z juice may be richer in vital minerals and vitamins necessary to facilitate yeast cellular adaptation to early efficient fermentations. Although SSV2Z juice has high sucrose contents similar to that of SSV2B juice, fermentation kinetics of both juices was different. However, the comparatively higher concentrations of group 1 amino acids, FAN and fermentable sugars present in the SSV2Z juice mean that yeast cells exhibit better fermentation performance in this medium compared with SSV2B which is comparatively lower in these nutrients. For bioethanol production, the correct nutritional balance for yeast in fermentation media is crucial in dictating ethanol yields [44-46]. Failure to sustain and maintain fast fermentation rates in KSV8Z after 24 h (see Figure 2) may be due to depletion of both fermentable sugar and assimilable nitrogen. Of the 4 juice substrates studied, SSV2B had the highest total group 2 amino acid content (as well as other amino acid groups), followed by SSV2Z and KSV8B juices (Table 3). The group 2 amino acids are assimilated at a steady rate by yeast during fermentation, whereas the other amino acid groups are normally assimilated towards the latter stages of fermentation. Regarding proline, the uptake of this amino acid may be virtually negligible depending on the "substrate's nutrient worth" [44].

Fermentation of the crude juice from SSV2 sorghum stalks (from site Z) resulted in the highest ethanol yield of over 80 g l^{-1} (Table 4). This compares favourably with the figure of 73 g l^{-1} reported by Gyalai-Korpos et al. [35] for crude juice from another

Table 4. Ethanol yield and residual FAN/sugars from fermented sorghum stalk juices.

Cultivar	SSV2		KSV8	
Location	Site B	Site Z	Site B	Site Z
Ethanol (g l^{-1})	65.26 ± 1.43^{a}	80.56 ± 2.17^{b}	36.31 ± 1.66^{c}	52.07 ± 0.81^{d}
FAN (mg L^{-1})	76.34 ± 1.46^{a}	89.13 ± 1.46^{b}	32.84 ± 1.46^{c}	17.83 ± 1.46^{d}
Sucrose (g l^{-1})	11.35 ± 1.04^{ab}	6.98 ± 0.31^{cd}	0	0
Glucose (g l^{-1})	0	0	0	0
Fructose (g l^{-1})	0	0	0	0
TOTAL sugars (g l^{-1})	11.35 ± 1.04^{ab}	6.98 ± 0.31^{cd}	0	0

Ethanol concentration yields and total residual sugars of sorghum stalk juice after fermentation. Results are Mean of triplicates ± SD. Results on the same row followed by different superscript letter (a-d) indicate significant difference ($p \leq 0.05$) by GLM (ANOVA) test.

sorghum cultivar, and the 86 g l^{-1} yield reported by Zhao et al. [39] for crude sorghum juices supplemented with additional nutrients (urea, DAP, and $MgSO_4$). In addition, the ethanol yields of all SSV2 and KSV8 crude juices, irrespective of cultivation site, also compared well within the range of 24-68 g l^{-1} reported by Widianto et al. [47] for a variety of crude juices from different sorghum cultivars fermented without nutrient supplementation. However, when compared to juices of other sorghum cultivars whose crude juices were pre-treated and enriched with cane molasses and other commercial yeast nutrients, ethanol from SSV2 juice fermentation did not compare favourably. For example, ethanol yields of 94 g l^{-1} to 130 g l^{-1} were reported by Nuanpeng et al. [29] and Yue et al. [38] in sugar-supplemented very high gravity (VHG) sorghum stalk juice fermentations.

Overall, fermentation of the SSV2 stalk juices, particularly those extracted from the cultivar grown in site Z (exemplified by colder and wetter conditions) resulted in the highest ethanol yields. This work therefore points to differences in the climatic conditions of sorghum cultivation significantly impacting on fermentation performance of stalk juices.

When ethanol yields are projected per hectare, the SSV2 sorghum cultivar crude juices in sites B and Z were estimated to reach 2062 l ha^{-1} and 2595 l ha^{-1} respectively (from Tables 2 and 4). These results compare well with the 2100-2345 l ha^{-1} results reported by Kothari et al. [48] and Serna-Saldivar et al. [49] for crude juices of various sorghum cultivars grown under rain-fed conditions and with agrochemical applications (in contrast to this study where SSV2 was cultivated under rain-fed conditions and no agrochemical application).The reported ethanol yields of 2062 l ha^{-1} and 2595 l ha^{-1} of crude juices of SSV2 sorghum in this study are lower than previously reported values of 3450-4132 l ha^{-1} for juices of sorghum crops cultivated under fertilizer and other agrochemical applications [13,49]. However, taking into account the potential economic and environment costs of the use of fertilizers and agrochemicals, ethanol yields reported in the present study appeared to be favourable in the wider context of responsible and manageable local bioresource utilisation and economic and environmental sustainability.

4. Conclusions

Choosing the right sorghum cultivar and suitable cultivation location may lead to significantly improved stalk juice ethanol yields. We recommend that sorghum crops destined for stalk juice fermentation should be cultivated in locations with good rain precipitation and moderate diurnal temperature. For example, the Nigerian SSV2 sorghum, despite grown under rain fed condition and without agrochemical applications, showed better ethanol yield potential in site Z with 600mm mean precipitation and 26.5^{o}C diurnal temperature. KSV8 appeared not to be economically viable juice source for bioethanol production. The potential of several other sorghum crops cultivated in Nigeria for biofuels deserves further attention.

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Bioethanol in Nigeria: comparative analysis of sugarcane and sweet sorghum as feedstock sources

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This review discusses the relative merits of sweet sorghum and sugarcane crops for the expanding bioethanol sector in Nigeria. We have compared, from a number of perspectives, sugarcane molasses and sweet sorghum stalk juice as biomass sources for Nigerian fuel alcohol fermentations and the findings indicate that sweet sorghum is most suited in terms of the adaptability of this crop to harsh climatic and cultivation conditions. In terms of environmental impact, sweet sorghum cultivation is more water efficient, requires less energy input, fertilization and agrochemical application. The concept of life cycle analysis was used to compare the environmental, social and economic impacts of using sweet sorghum stalk juice and sugarcane molasses. Sweet sorghum represents a more favourable biomass source and there is great potential for sustainable development and utilization of sweet sorghum for bioenergy production in Nigeria. However, there is need for well defined, structured, coordinated, targeted and monitored scientific efforts and investments in order to realize maximum associated benefits.

Introduction

With increasing future global demand for fossil fuels, oil price fluctuations and future supply availability, together with climate change concerns, renewable energy sources are receiving widespread attention.[1] Biofuels such as bioethanol, biodiesel and biogas are essential contributors to this resurgence in demand for alternative energy. For bioethanol, agriculturally derived starch and sucrose are the predominant fermentation feedstocks[2] and the world's major producers are, respectively, USA (using maize) and Brazil (using sugarcane).

In Nigeria, commercial bioethanol production was initiated in 1972 when the Nigerian Government received a business proposal from Volgelbutsch of Austria to utilise the waste cane molasses from the premier sugar company (Nigerian Sugar Company) located in Bacita, Kwara state. Consequently, the Nigerian Yeast and Alcohol Manufacturing PLC (NIYAMCO) was commissioned in 1973. NIYAMCO's operation depended solely on the availability of cane molasses as a feedstock from the sugar company. Unfortunately, the sugar company collapsed in 1994 seriously affecting NIYAMCO's activities. This meant that an alternative feedstock for bioethanol production was needed for the ethanol plant, but unfortunately, due to financial difficulties, NIYAMCO closed down in 1999, although cassava crop was initially identified as the alternative feedstock.[3]

In Nigeria, the agricultural sector is large,[4] and it is envisaged that biofuel (particularly bioethanol) development will attract private investment to improve agricultural practices, accelerate rural development and create wealth.[5] This review provides a comparison between sugarcane and sweet sorghum as energy crops for future Nigerian bioethanol production.

Bioethanol production in Nigeria

Currently, Nigerian bioethanol is largely used for blending with gasoline to produce E10 transport fuel and to a lesser extent is mixed with thickening agents such as cellulose to produce Gelfuel normally used for cooking.[6] Domestic demand in Nigeria for bioethanol E10 blends was estimated to be 180 million litres in the year 2005, but all of this alcohol was imported.[7]

Sugarcane and sweet sorghum are crops which provide sugars (*i.e.* sucrose, glucose, fructose, *etc.*) that are suitable for bioethanol production, as they are readily fermentable by the yeast, *Saccharomyces cerevisiae*. Together with cassava, these are considered most suitable Nigerian bioethanol feedstocks.[8] Nevertheless, there are several factors constraining biofuel developments in Nigeria, not least its failure to be fully self-sufficient in feeding its teeming population, which is projected to be the 4th largest in the world by 2050.[9] Due to such food security issues, the use of cassava and sugarcane as energy crops has

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Broader context

Sweet sorghum (stalk juice) and sugarcane (molasses) have potential as biomass sources for the expanding bioethanol sector in Nigeria. Our analyses shows that, from a number of environmental, social and economic perspectives, sweet sorghum is most suited for Nigerian fuel alcohol fermentations mainly due to the adaptability of this crop to harsh climatic and cultivation conditions. In terms of environmental impact, sweet sorghum cultivation is more water efficient and requires less agrochemicals than sugarcane.

attracted criticism. For example, Nigerian sugarcane is unable to satisfy local demand for refined sugar consumption, which is almost wholly dependent on importation.[10] Thus, sweet sorghum has been viewed as the "promising feedstock crop" for bio-ethanol production.[11]

Sweet sorghum in Nigeria has no significant commercial utilisation, with only the grain being used as source of starch for the brewing industry and for the production of non-alcoholic malt drinks. The stalk from a certain variety called 'Takanda' is normally chewed as an alternative to sugarcane, while the residue is used as animal feed. Hence, more than 90% of the 9.24 million tonnes of sweet sorghum crop cultivated in Nigeria[12] is directly consumed without undergoing any significant commercial processing.[13] The demand for sorghum for human consumption is rapidly dwindling due to the availability of other preferred cereals such as rice so there is great potential for sorghum to be exploited as a Nigerian energy crop. Importantly, about 85% of arable land in Nigeria (785 000 km^2) can support sweet sorghum

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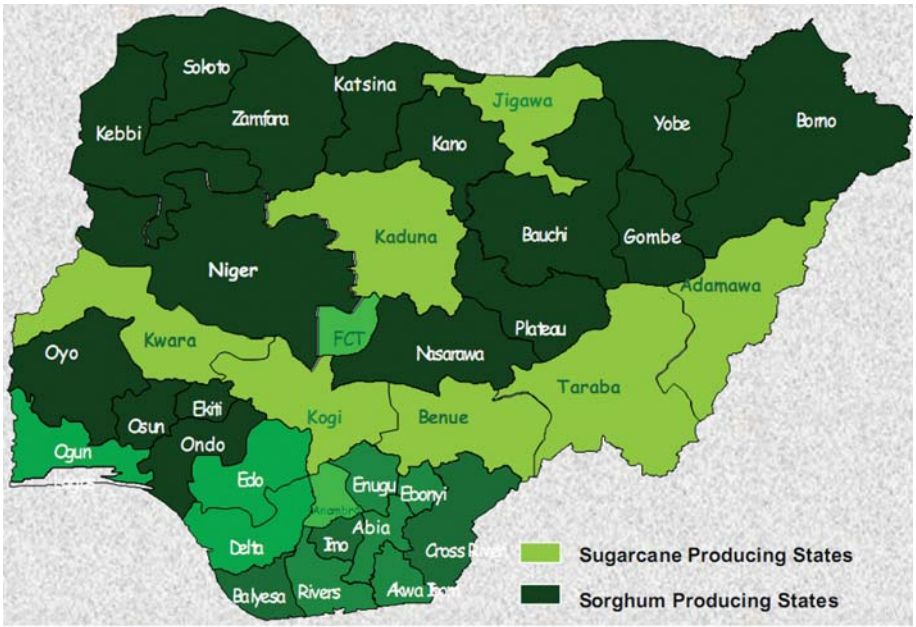

Map of Nigeria


Sugarcane crop


Sweet sorghum crop


Sweet sorghum crop

cultivation, with only 52% being currently used for agricultural activities.[9]

Considering sugarcane, this is principally a tropical crop that thrives best under warm climatic conditions and with adequate rainfall. It is cultivated in Nigeria for refined sugar production.[14] By comparison, sweet sorghum has wider adaptability range, can tolerate drought or waterlogged conditions in both tropical and temperate regions.[15] Interestingly, sorghum exhibits resistance to attack by *Striga*,[16,17] which is a hemi-parasitic weed that infests cereal crops resulting in great economic losses.[18,19] As with sugarcane, sweet sorghum stalk juice predominantly contains sucrose but it additionally comprises relatively higher concentrations of other sugars such as glucose, fructose and maltose rendering it unsuitable for refined sugar production. Furthermore, sweet sorghum bagasse is considered to have higher biological value as animal feedstock relative to cane bagasse.[15]

Nigerian sugarcane cultivation

There are two types of sugarcane crop commonly cultivated in Nigeria: chewing cane (soft cane) and industrial cane. The former is mainly produced by local farmers and is chewed raw for its sweet sucrose-rich juice that is also commonly processed into 'Mazarkwaila' (a local substitute for sugar) and 'Alewa' (a local sweet snack).[20] The industrial cane is composed of hard biomass fibre and is mostly produced commercially by sugar estates for refined sugar production[20] and the by-product of the sugar refining process (*i.e.* molasses) may be utilized for bioethanol production.[21] Sugarcane is cultivated on 25 000–30 000 hectares of Nigerian land, with the industrial cane covering only about 12 000 hectares, and soft cane covering the remaining available land.[22] The soft cane has higher price per tonne in Nigeria than the industrial cane, therefore, it attracts more commercial interest to local farmers than industrial cane.[20] Nigeria produces only 50 000 tonnes of sugar out of a total consumption of 1.176 million tonnes, annually.[10] This has necessitated the creation of a National Sugar Development Council (NSDC) and facilitated the building of five new sugar plants to boost sugar production, as well as to enhance the nation's capacity for the generation of alternative renewable energy from sugarcane molasses.[10]

The major constraint limiting sugar yields in production of both chewing and industrial sugarcane crops in Nigeria is smut disease, caused by the pathogen *Ustilago scitaminea Syd*.[22] Other challenges faced by local sugarcane farmers are poor soil nutrition and weeds. Specifically, there is inadequate availability of inorganic fertilizer to supplement soil nutrients, particularly during the second and third crop rattooning. Excessive weed growth coupled with poor soil nutrition leads to poor stalk yield, reduced cane weight, high fibre content and poor juice quality at harvesting.[23] Modern methods of weed control by herbicides are limited by supply and affordability to local farmers, who resort to the use of crude, laborious and time-consuming hand hoeing to effect control.[23,24] The following cultivation and harvesting factors relate to production of 1 tonne sugarcane crop:

• Water requirement for annual sugarcane crop cultivation is estimated to be 36 000 m^3 ha^{-1}.[25] This is equivalent to 514.29 m^3 per tonne cane cultivated at sugarcane crop yield of 70 t ha^{-1}.[26]

• Typically, freshly harvested industrial cane variety, such as Nigerian KD-10 variety has an estimated 20.6% Brix sugar content[22] with average sucrose content of fresh stalk juice being 14%.[21]

• The average industrial grade sugarcane crop yield per hectare of land cultivated is about 70 t ha^{-1}.[26] Typical fertilizer application rates for P, K, and N elements are:[14] 80 kg ha^{-1} of P_2O_5 (*i.e.* superphosphate), 60 kg ha^{-1} of K_2O (*i.e.* muriate of potash) and 120 kg ha^{-1} of N from urea respectively.

• Thus, for an average yield of 70 t ha^{-1}, fertilizer consumption per tonne of sugarcane crop cultivated is estimated to be 1.14, 0.86 and 1.71 kg of superphosphate, muriate of potash and nitrogen respectively.

• It has been shown that 2 kg ha^{-1} herbicides (Atrazine or Diuron) may be consumed for the cultivation of sugarcane at crop yield of 63 t ha^{-1} corresponding to 0.03 kg herbicide.[23] Furthermore, 0.85 kg ha^{-1} of insecticide (Monocrotophos) is equally consumed on crop yield of 39 t ha^{-1} cane *i.e.* 0.02 kg insecticide per t.[14]

• A tonne of freshly harvested sugarcane crop is estimated to generate a total of 280 kg of green trash, comprising of cane tops, leaves, roots, defective cane stalk, *etc.*[27,28] This implies that 720 kg of millable cane are obtainable per tonne of cane harvested.

The characteristics of sugarcane described above are summarised in Table 1.

Nigerian sweet sorghum cultivation

Sorghum is regarded as an important cereal crop in Nigeria covering more than 45% of the total land devoted to cereal production in the country.[24] Several cultivars are grown entirely in the Savannah and Sahelian region of Northern Nigeria, which constitute over 67% of the Nigerian landmass of 923 768 km^2 and where the natural geographic conditions (semi-arid or dry tropics) are the most suited for optimal sorghum yield. Nigeria accounts for over 71% of sorghum production in West Africa[17] and is ranked as the 2nd largest producer in the world with over 9.2 million tonnes per annum.[12]

Sweet sorghum stalk juice may be used in the production of bioethanol while the grain is primarily used as a human and animal food source. Sorghum grain may also be processed to produce traditional alcoholic drinks such as 'burukutu' and 'tala', or non-alcoholic drinks such as 'kunun-zaki'.[29] However, there is increasing concern regarding sustainability of large scale sorghum production in Nigeria due to prevalence of sorghum infestation by *Striga* (a parasitic weed), although sorghum cultivars such as *Kaura*, and *Farafara* are tolerant to this parasite.[16,17,30]

Other factors influencing Nigerian sorghum crop yield and quality are tillage and soil fertility. Furthermore, inorganic fertilizers and herbicides are scarcely available and affordable to local farmers who are compelled to resort to using cattle dung and poultry manure as alternative high-nitrogen fertilizers.[24,31] Yield losses of 40–80% have been attributed to weed competition during cultivation of sorghum crop. An overview of the cultivation and harvesting of sorghum crop is outlined below:

• The grain and millable stalk yield of sweet sorghum largely depend on crop variety, soil condition and cultivation practice.[17]

• The average sweet sorghum crop yield in sub-Saharan Africa, at 2 crop cycle per annum, is about 92 t ha^{-1}. The average sorghum grain and millable stalk yield are 5 t ha^{-1} and 70 t ha^{-1}, respectively, while the green trash (*i.e.* leaves, roots, straw, defective stalks, *etc.*) is 17 t ha^{-1}.[32]

• Thus, it may be deduced that 1 tonne of whole fresh crop harvested will yield 54.35 kg grain (*i.e.* $5/92 = 0.05435$ t $\times$ 1000 = 54.35 kg), 760.87 kg millable stalk and 184.78 kg green trash (*i.e.* $17/92 = 0.18478$ t $\times$ 1000 = 184.78 kg).

• Freshly harvested sweet sorghum stalk juice has a minimum of 12% sucrose content[33] and about 18.7% Brix extracted juice sugar content.[32]

• The annual water requirement for cultivation of sweet sorghum crop over 2 crop cycles is estimated to be 8000 m^3 per annum.[25] Considering that the crop yield is about 92 t ha^{-1},[32] thus water consumption can be estimated to be about 86.96 m^3 per tonne crop cultivated.

• Typically, fertilizer application rate on relatively normal fertile soil for sweet sorghum crop cultivation is estimated as 60, 20, and 33 kg for nitrogen, phosphorus, and potassium,

Table 1 Summary of resource input for the cultivation of sugarcane and sweet sorghum crops

S/No	Item	Sugarcane	Sorghum
1	Crop yield per cropping cycle/t ha^{-1}	70	46
2	Cropping cycle per annum	1	2
3	Harvest (months after planting)	12	4
4	Cultivation water consumption/m^3	514.29	86.96
5	Fertilizer consumption, N : P : K/kg	1.71 : 1.14 : 0.86	1.30 : 0.44 : 0.72
6	Herbicide consumption/kg	0.03	0.01
7	Insecticide consumption/kg	0.02	—
8	Fresh juice sugar content (% Brix)	20.6	18.7
9	Stalk juice sucrose content (% w/w)	14	12
10	Millable cane yield/kg	720.00	760.87
11	Green trash yield/kg	288.00	184.78
12	Grain yield/kg	—	54.35

respectively, per hectare per 1 crop cycle.[34,35] Knowing that the crop yield is about 92 t ha^{-1}, hence fertilizer consumption per tonne of yield in 1 crop cycle can be estimated to be 0.435 kg P_2O_5, 0.72 kg K_2O and 1.30 kg N.

• It has been reported that the application of 0.05 kg a.i. ha^{-1} Cinosulfuron, a chemical herbicide, during cultivation of sweet sorghum crop, is necessary for effective control of weed attack and improved grain yield.[36] This is equivalent to 0.001 kg herbicide consumption per 1 tonne of sorghum crop cultivated.

Table 1 provides summaries of the resource input for both crops on one tonne basis.

Energy balance

It is important in selecting any bioenergy platform that the net energy balance is highly positive, in order to achieve and maintain optimal economic dividends and environmental sustainability. This forms a specifically quintessential part of the informed decisions needed in considering which crops and bioenergy conversion strategies are worthy of biotechnological investment. A summary of the energy consumed in the production of fertilizer and chemical use in the cultivation of 1 tonne of either sugarcane or sweet sorghum crop is provided in Table 2 and the corresponding green house gas (GHG) emissions from the production of fertilizers and chemicals are shown in Table 3.

Tables 1–3 indicate that, in comparison with sugarcane, sweet sorghum is relatively less expensive to grow, produces higher yields, with lower fertilizer and herbicide inputs, and with very limited water consumption per tonne of crop. This is consistent with previous studies that compared sorghum with sugarcane and with other energy crops such as sugar beet, maize and soybean.[39–41]

Energy consumption and GHG emission from sugarcane cultivation

The estimated average energy requirement for cultivation and harvesting of sugarcane is 30.10 MJ per tonne raw cane.[42] An estimated 83% of this total energy is for diesel fuel and gasoline required for running agricultural machines and farm vehicles, and the remainder for electricity (needed for irrigation, lighting, *etc*).[38] The total energy consumption for cultivation and harvesting of 1 tonne sugarcane crop is summarised in Table 4, and the corresponding GHG emissions are given in Table 5.

Energy consumption and GHG emission from sweet sorghum cultivation

Tables 4 and 6 show that the energy requirement for cultivation and harvesting of 1 tonne sweet sorghum crop is about 50% that of sugarcane under fully mechanised farming practice.[46] Data for corresponding GHG emissions are presented in Table 7.

Data presented in Tables 4–7 clearly show that sweet sorghum exhibits distinct energetic advantages over sugarcane (50% less energy input) and also generates about half the total GHG emissions in the form of CO_2. Therefore, sorghum represents

Table 2 Energy consumption for the production of fertilizer and agro-chemical use in cultivation of Nigerian energy crops

Item	Sugarcane cultivation			Sweet sorghum cultivation		
Fertilizer/ chemical	Mass input/kg t^{-1} cane	Energy input[a]/MJ kg^{-1}	Total energy input[b]/MJ t^{-1}	Mass input/kg t^{-1} cane	Energy input[a]/MJ kg^{-1}	Total energy input[b]/MJ t^{-1}
Urea, N	1.71	69.5	118.845	1.30	69.50	90.350
Phosphate	1.14	7.7	8.778	0.44	7.70	3.388
Potassium	0.86	6.4	5.504	0.72	6.40	4.608
Insecticide	0.02	358.0[c]	7.160	—	358.0[c]	—
Herbicide	0.03	355.6[c]	10.668	0.01	355.6[c]	3.556
Total			150.955			101.902

[a] The respective energy consumption values per kilogram of fertilizer produced in Nigeria as adopted from literature.[37] [b] The evaluated total energy input (in terms of fertilizer production) per tonne of crop cultivated (*e.g.*, 1.71 kg t^{-1} cane × 69.5 MJ kg^{-1} N = 118.845 MJ t^{-1} cane) *etc*. [c] Reported energy requirements for production of insecticide and herbicide are 358.0 MJ kg^{-1} and 355.6 MJ kg^{-1} respectively.[38]

Table 3 GHG emissions during the production of fertilizer and agrochemicals used in cultivation of Nigerian energy crops

Item	Sugarcane cultivation			Sweet sorghum cultivation		
Fertilizer/chemical	Mass input/kg t^{-1} cane	Emission factor[a]/kg CO_2 eq per kg	Total emission[b]/kg CO_2 eq t^{-1} cane	Mass input/kg t^{-1} cane	Emission factor[a]/kg CO_2 eq per kg	Total emission[b]/kg CO_2 eq t^{-1} cane
Urea, N	1.71	3.97	6.789	1.30	3.97	5.161
Phosphate	1.14	1.30	1.482	0.44	1.30	0.572
Potassium	0.86	0.71	0.611	0.72	0.71	0.511
Insecticide	0.02	29.00	0.580	—	29.00	—
Herbicide	0.03	25.00	0.750	0.01	25.00	0.250
Total			10.212			6.494

[a] IPCC GHG emission factor for production of agrochemicals.[38] [b] Total emission from the production and application of agrochemicals for each crop cultivation (*e.g.* for urea consumed in 1 tonne cane cultivation, the total emission is $1.71 \times 3.97 = 6.789$ kg CO_2 eq t^{-1} cane) *etc.*

a bioenergy crop with promising potential from both energy and environmental perspectives.[39–41]

Sugarcane molasses as bioethanol fermentation feedstock

Typical Nigerian sugar refining processes produce an average of 82.2 kg refined sugar and 31 kg molasses per tonne of sugarcane harvested.[47] Furthermore, the total fermentable sugar content of molasses is around 46%[21,48] meaning that 31 kg molasses will typically have 14.26 kg fermentable sugar content. Hence, it can be deduced from the stoichiometric eqn (1) that 14.26 kg fermentable sugar ($C_6H_{12}O_6$) will yield 7.29 kg of ethanol (C_2H_5OH).

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \quad (1)$$

The estimated industrial fermentation efficiency is 92%,[21] translating to an ethanol yield of 6.71 kg or 8.50 litres (with specific density of 789.4 kg m^{-3} for ethanol). If industrial distillation efficiency is about 97%,[48] ethanol yields can be estimated to be 8.25 litres from 31 kg of molasses. The average yield estimate in the USA is 241.8 litres of ethanol per tonne molasses fermented,[49] which is also equivalent to 7.50 kg per 31 kg molasses. Finally, theoretical CO_2 yield from yeast (*S. cerevisiae*) fermentation (eqn (1)) is 0.489 kg. Therefore 14.26 kg of fermentable sugar would produce 6.97 kg CO_2 gas at 92% fermentation efficiency, resulting in 6.42 kg total CO_2 emission for 31 kg of raw molasses.

Sweet sorghum juice fermentation to ethanol

Fermentable sugar content of fresh sorghum juice is estimated to be 18.7% and extractable juice yields 41.2% of the millable stalk weight.[50] Therefore, 760.87 kg millable sweet sorghum cane can produce 313.48 kg juice, while the total fermentable sugar content will amount to 58.62 kg. Using the stoichiometric eqn (1) and similar calculation steps as above, the theoretical ethanol yield in this case is estimated to be 29.96 kg (C_2H_5OH) from 760.87 kg of millable sweet sorghum. At 85% industrial fermentation efficiency[15] the ethanol yield becomes 25.47 kg (C_2H_5OH) or 32.26 litres. Considering the 97% industrial distillation efficiency,[48] the industrial scale ethanol yield becomes 31.29 litres. Average yield estimates of 30–40 litres of ethanol per tonne sweet sorghum crop have been reported.[51] The theoretical volume of CO_2 gas produced during fermentation of 58.62 kg of sweet sorghum juice is 28.67 kg, which at 85% fermentation efficiency the total CO_2 emission becomes 24.37 kg per 760.87 kg of millable sweet sorghum.

Table 4 Energy input for sugarcane harvesting and cultivation[a]

Fuel type	Fuel share (%)	Energy input/MJ t^{-1} cane
Diesel gas (A.G.O)	62.47	18.80
Gasoline (PMS)	20.73	6.24
Electricity	16.80	5.06
Total		30.10

[a] Data sources: deduced values from ref 38 and 42.

By-products of ethanol production from sugarcane and sweet sorghum

The environmental impact of ethanol production from bioenergy crops, such as sugarcane or sweet sorghum, can be considerably high because of the large amounts of stillage[52–54] that often escape into waterways,[55] and crushed stalks or bagasse produced.[56–59]

Stillage

Molasses-based distilleries are one of the most polluting industries generating large volumes of wastewater, vinasse or stillage.[53–55] Stillage is a low-solids liquid waste whose pollution load index is generally characterised by a high biological oxygen demand (BOD) and chemical oxygen demand (COD).[52–54] An estimated 10–20 litres of stillage is generated per litre of ethanol produced.[52,54,60] Therefore, based on previous projections and extrapolations (see section above on Sugarcane molasses and eqn (1)), molasses ethanol from 1 tonne sugarcane may generate 165 kg stillage, while sorghum juice ethanol from 1 tonne sweet sorghum may generate 625.8 kg stillage. In Nigeria, large amounts of stillage are discharged into waterways without effective proper treatment.[55] Future developments may employ physical, mechanical, chemical, and biological treatment technologies that aim to turn stillage into animal feed, biogas, fertilizers and other renewable chemical products.[31,52–54,61] For example, the use of stillage as a fertilizer in sugarcane fields has

Table 5 Sugarcane cultivation energy input and corresponding GHG emission

Fuel type	Energy input/MJ t^{-1} cane	Emission factors	Total emissions[d]	GWP[c]	Emissions/kg CO_2 eq t^{-1} cane
Diesel gas	18.80	0.0741 kg CO_2 MJ^{-1}[a]	1.393 kg CO_2 t^{-1} cane	1	1.393
Gasoline	6.24	0.0693 kg CO_2 MJ^{-1}[a]	0.432 kg CO_2 t^{-1} cane	1	0.432
Electricity	5.06	0.103 kg CO_2 MJ^{-1}[b]	1.521 kg CO_2 t^{-1} cane	1	1.521
		0.000004 kg CH_4 MJ^{-1}[b]	0.00002 kg CH_4 t^{-1} cane	25	0.0005
		0.0000005 kg N_2O MJ^{-1}[b]	0.000003 kg N_2O t^{-1} cane	298	0.0009
Total	30.10				3.347

[a] IPPCC default GHG emission factor values for fossil fuel combustion.[43] [b] GHG emission factor for electricity consumption in Nigeria.[44] [c] Global Warming Potentials for gas species.[45] [d] Total emission is the cumulative emission from use of each fuel type (*e.g.* diesel gas = 18.80 MJ t^{-1} cane × 0.074 kg CO_2 MJ^{-1} = 1.393 kg CO_2 t^{-1} cane) *etc.*

Table 6 Energy input for sweet sorghum cultivation[a]

Fuel type	Fuel share (%)	Energy input/MJ t^{-1} cane
Diesel gas (A.G.O)	62.47	9.40
Gasoline (PMS)	20.73	3.12
Electricity	16.80	2.53
Total		15.05

[a] Data source: deduced values from ref 42 and 46.

been reported to increase productivity by 20–30%.[62] This approach is highly relevant and applicable in Nigeria, especially in the context of the general dearth and prohibitive cost of fertilizers. Ethanol production from thin stillage using genetically engineered microorganisms[63] represents biotechnological innovation and a tempting national challenge.

Filter cake

Filter cake results from the concentration and drying of stillage or vinasse to reduce their moisture content. About 35 kg of filter cake are generated per 1 tonne of cane/stalk milled.[28] Thus, for 720 kg millable cane, 25.20 kg filter cake will be produced, while for 760.87 kg millable sorghum stalk, 26.63 kg filter cake will be generated. This is a major potential source of additional bioenergy in the form of compost (organic–mineral fertilizers) to support crop production,[31,57] which can be exploited in Nigeria.

Bagasse yield and energy generation

Crushed stalks (bagasse) are by-products of the cane milling process, which together with the stripped leaves of the sweet sorghum, can be compacted into nutritious blocks as animal feeds.[64,65] The bagasse obtained after juice extraction from the stalks contains a concentrated amount of cellulose, which is also a potential source of ethanol.[59,65–69] Such approaches are economically more viable with sweet sorghum bagasse compared with bagasse from other biomass sources. Recent research efforts worldwide are leading to cellulosic bioethanol becoming commercial reality.[69–73] Further, composting and anaerobic digestion technologies can be applied to produce biogas from bagasse.[59,66–68] It is of significant interest that sweet sorghum bagasse can serve as solid support matrix for immobilizing yeast (*S. cerevisiae*) in a stable bioreactor for ethanol fermentation from sucrose.[68] Sugarcane bagasse has also been considered as a potential adsorption matrix for the remediation of metal ions from aqueous wastes.[74] It is tempting to consider the feasibility of the use of sweet sorghum bagasse in similar application. However, the most common usage is the combustion of bagasse to generate heat and electrical energies.[56–58,75]

While average sweet sorghum bagasse yield may be estimated as 280 kg per tonne green stalk harvested,[76] the sugarcane crop bagasse is estimated at 202 kg per tonne fresh crop harvested.[77] Assuming that all the green trash is utilized along with the bagasse for combustion, thus:

- Total cane bagasse equivalent = 288 kg + 202 kg = 490 kg (at 50% moisture content).
- Total sorghum bagasse equivalent = 280 kg + 184.78 kg = 464.78 kg (at 50% moisture content).

Projecting 10% losses of bagasse being equivalent to transportation and handling prior to boiler combustion will result in 441 kg and 418.30 kg bagasse from 1 tonne sugarcane and sorghum, respectively. Whilst the lower heating value (LHV) for cane bagasse (at 50% moisture content) is reported as 7.868 MJ kg^{-1},[78] the LHV value for sorghum bagasse equivalent is estimated at 7.633 MJ kg^{-1} (at 50% moisture content).[79] Biomass

Table 7 Corresponding GHG emission from sweet sorghum cultivation

Fuel type	Energy input/MJ t^{-1} cane	Emission factors (EF)	Total emissions	GWP[c]	Emissions/kg CO_2 eq t^{-1} cane
Diesel gas	9.40	0.0741 kg CO_2 MJ^{-1}[a]	0.697 kg CO_2 t^{-1} cane	1	0.697
Gasoline	3.12	0.0693 kg CO_2 MJ^{-1}[a]	0.216 kg CO_2 t^{-1} cane	1	0.216
Electricity	2.53	0.103 kg CO_2 MJ^{-1}[b]	0.261 kg CO_2 t^{-1} cane	1	0.261
		0.000004 kg CH_4 MJ^{-1}[b]	0.00001 kg CH_4 t^{-1} cane	25	0.0003
		0.0000005 kg N_2O MJ^{-1}[b]	0.000001 kg N_2O t^{-1} cane	298	0.0004
Total	15.05				1.175

[a] IPPCC default GHG emission factor values for fossil fuel combustion.[43] [b] GHG emission factor for electricity consumption in Nigeria.[44] [c] Global Warming Potentials (GWPs) for gas species.[45]

fuelled boiler efficiency is typically around 79%.[38] Hence, the projected totals of renewable energy output from bagasse combustion for each crop (assuming same LHV value for both bagasse equivalents) are as follows:

(i) Projected renewable energy output from cane bagasse equivalent per tonne sugarcane = 7.868 MJ kg^{-1} × 441 kg × 0.79 = 2741.13 MJ. However, the Brazilian average estimate for year 2006 is 2198 MJ t^{-1} cane.[38]

(ii) Projected renewable energy output from sorghum bagasse equivalent per tonne crop = 7.633 MJ kg^{-1} × 418.30 kg × 0.79 = 2522.38 MJ.

The only significant GHG emissions from bagasse fuelled controlled boilers are particulate matters (VOC) and nitrous oxide (N_2O) respectively.[42] A summary of GHG emissions from the burning of both sugarcane and sweet sorghum bagasse in boilers for energy generation is presented in Table 8, and a summary for total energy input for production of bioethanol from 1 tonne of sweet sorghum and sugarcane crop and the cumulative corresponding GHG emissions from use of the energy is presented in Table 9. For example, total N_2O emission from cane bagasse burning = 0.00002 kg N_2O × 441 kg bagasse = 0.00882 kg N_2O, or 0.00882 kg N_2O × 298 (GWP) = 2.628 kg CO_2 eq t^{-1} cane. Bioethanol production processes can thus be energy self-sufficient, since the input energy requirements for cane milling and other operations are supplied by burning of bagasse, and the surplus energy exported to the national grid.[57,58,75,80] Considering fossil fuel energy input in ethanol production, an estimated 45.4 MJ t^{-1} millable cane is consumed in distillery operations[81] and 13.90 KWh t^{-1} millable cane (*i.e.* 50.04 MJ t^{-1}) is required for cane milling.[80] Hence, energy demand per millable cane or stalk can be estimated as follows: (i) 720 kg millable cane requires = 720 kg × 0.05004 MJ t^{-1} = 36.03 MJ energy for cane milling and 720 kg × 0.0454 MJ t^{-1} = 32.69 MJ energy for fermentation and distillation and (ii) 760.87 kg millable stalk requires = 760.87 kg × 0.05004 MJ t^{-1} = 38.07 MJ energy for stalk milling and 760.87 × 0.0454 = 34.54 MJ energy for fermentation and distillation.

Energy ratios

The total calculated energy input for molasses bioethanol production from sugarcane crop is 249.775 MJ t^{-1} cane, while total calculated renewable energy output from cane bagasse burning is 2741.13 MJ t^{-1} cane.

Hence, the output/input energy ratio for molasses bioethanol = 2741.13/249.775 = 11.

However, the energy ratio for sorghum juice bioethanol = 2522.38/189.562 = 13.

It is expedient to mention that the energy input in the production of fossil fuels used during crop cultivation, machinery and equipment manufacturing energy requirements, and energy requirements for transportation and distribution of the anhydrous bioethanol are not considered in this review.

Furthermore, energy output from refined sugar consumption, stillage and bioethanol combustion is not equally considered here. Nevertheless, this is an important cue for Nigeria to utilise bagasse for power generation, since stable production and supply of power have remained a challenge in Nigeria.

Although both sugarcane and sweet sorghum crops may be considered as sustainable feedstock sources for bioethanol production in Nigeria, using life cycle analysis concepts to compare bioethanol production from cane molasses and sorghum stalk juice indicates the following (as summarised in Table 10).

1. If there is concern regarding energy crops competing for cultivable land with food crops, sweet sorghum with relatively higher crop yield of 92 t ha^{-1} will be more favourable than sugarcane crops which have about 70 t ha^{-1} (higher crop yield per hectare implies optimum land utilisation).

2. Cane molasses (obtained from 1 tonne raw sugarcane crop) produces approximately one-quarter volume of bioethanol that may be produced from direct use of sorghum stalk juice per tonne of raw crop processed, though it may equally be argued that the direct use of sugarcane stalk juice may also give higher bioethanol volume yield per tonne crop than sorghum juice, the issue of refined sugar production from sugarcane juice may be said to be relatively paramount in Nigeria.

3. Although the gross energy input to produce 1 litre of bioethanol from sweet sorghum crop is about one-fifth of the gross energy requirement to produce a litre from sugarcane crop (which makes sorghum look like a more favourable feedstock), the energy output from burning of sorghum bagasse in a boiler is about one-quarter of the energy output from burning sugarcane bagasse per litre of bioethanol produced. Thus, considering the prevailing power shortage problem faced by Nigeria, sugarcane crop may be a relatively more favourable feedstock for bioethanol production, if the bagasse will be utilized as an alternative source for additional power generation.

4. The cumulative estimated water consumption per litre of bioethanol produced from sorghum crop feedstock is marginally negligible compared to that of sugarcane crop. Therefore, for a country like Nigeria facing water supply challenges in the agricultural sector, relying on sugarcane crop as feedstock source

Table 8 GHG emission from bagasse boilers

		Sugarcane bagasse				Sorghum bagasse	
EF per kg[a] bagasse burnt	GWP	Mass/kg	Emission per species/kg	Emissions/kg CO_2 eq t^{-1} cane	Mass/kg	Emission per species/kg	Emissions/kg CO_2 eq t^{-1} cane
0.00002 kg N_2O	298	441	0.00882	2.628	418	0.00836	2.491
0.0000087 kg VOC	10	441	0.00384	0.038	418	0.00364	0.036
Total				2.666			2.527

[a] Emission factor (EF) per kg bagasse burnt.[42]

Table 9 Energy input summary

Item	Sugarcane		Sweet sorghum	
	Energy input/MJ	GHG emissions/kg CO_2 eq	Energy input/MJ	GHG emissions/kg CO_2 eq
Agrochemicals	150.955	10.212	101.902	6.494
Crops cultivation	30.10	3.347	15.05	1.175
Milling operations	36.03	—	38.07	—
Fermentation/distillation	32.69	—	34.54	—
CO_2 from fermentation	—	6.42	—	24.37
Total	249.775	19.979	189.562	32.039

for bioethanol production will further constrain adequate water supplies in the agricultural sector.

5. With regard to environmental sustainability, large scale sugarcane crop cultivation in Nigeria may pose more environmental challenges due to relatively higher consumption of fertilizer and herbicides/insecticide when compared to sweet sorghum crop. Sorghum crop per cultivable hectare requires less than one-fifth of total agro-chemicals required to cultivate a hectare of sugarcane. Application of high dosage of agro-chemicals for cultivation of crops without diligent consideration for environmental pollution control may lead to pollution of surface and groundwater which in turn may cause loss of species biodiversity.

6. Furthermore, concern about climate change and the desire of the Nigerian Government to benefit from carbon credits through reduced carbon emission indicates that sorghum crops represent better sustainable feedstocks for bioethanol production relative to sugarcane, because as indicated in Table 10, GHG emissions from production of bioethanol from sweet sorghum juice is less than one-third that of cane molasses on per litre basis.

7. Although stillage produced from production of bioethanol is the same per litre of ethanol produced from both crops, about two-third volume less of filter cake is produced from sorghum bioethanol. Therefore, when sorghum bioethanol filter cake is not utilized to serve as animal feed, it may constitute 'less' of an environmental threat.

Comparative economic feasibility of sugarcane *versus* sweet sorghum for Nigerian bioethanol production

For a typical bioethanol plant, feedstock costs typically account for about 55–75% of total variable costs associated with production operations.[92] Thus, feedstock costs substantially affect the overall economics of bioethanol production and the use of low cost feedstock will lead to favourable market returns.[93] Sugarcane is envisaged to be an unfavourable feedstock for Nigerian bioethanol largely due to the cost of irrigation water required during its cultivation when compared to sweet sorghum.[32] For sugarcane, water cultivation requirements have been estimated at 800 mm ha^{-1}, or about 1333 litres of irrigation water per litre of ethanol produced (*i.e.* at 50% irrigation efficiency).[94] However, sweet sorghum, like most other cereal crops, responds to irrigation more at certain growth stages (*i.e.* booting, flowering and grain fill stages) whilst at other stages, such as early vegetative and drought growth stages, the water requirement is minimal.[95] In fact, sorghum crops are commonly grown under rain-fed conditions in sub-Saharan Africa using traditional farming systems and in Nigeria irrigation water is rarely used in their cultivation.[85]

The average cost of irrigation development in Nigeria has been estimated at $13 500 per hectare with additional annual running costs for pumping operations and maintenance at around $280 ha^{-}.[96] Thus, additional investment costs for cultivation of sugarcane under irrigation conditions will require a further $13 780 ha^{-}, which further increases overhead costs of bioethanol production from sugarcane. It has been estimated that the total cost of sugarcane cultivation with irrigation water is $995 ha^{-} which is substantially greater than that of cultivating sweet sorghum crop with irrigation water which has been estimated at $238 ha^{-}. Without irrigation, sorghum crop cultivation has been estimated at $220 ha^{-}.[51] Hence, the average cost of sorghum bioethanol is around $1.74 per gallon (*i.e.* 3.78 litres) compared with that of sugarcane bioethanol at around $2.19 per gallon.[97]

Table 10 Overall summary

Item	Sugarcane	Sweet sorghum
1. Annual raw crop yield/tonnes $hectare^{-1}$	70	92
2. Ethanol production per tonne of raw crop/litres $tonne^{-1}$	8.25	31.29
3. Energy input per litre of ethanol produced/MJ $litre^{-1}$	30.28	6.06
4. Energy output from bagasse burning per litre of ethanol produced/MJ $litre^{-1}$	332.26	80.61
5. GHG emission per litre of ethanol produced/kg CO_2 eq $litre^{-1}$	2.42	0.99
6. Water consumption per litre of ethanol produced/m^3 $litre^{-1}$	62.3	2.80
7. Fertilizer consumption per litre of ethanol produced, N : P : K/g $litre^{-1}$	207 : 138 : 104	42 : 14 : 23
8. Herbicide/insecticide consumption per litre of ethanol produced/g $litre^{-1}$	6.0	0.3
9. Stillage production per litre of ethanol produced/kg $litre^{-1}$	20	20
10 Filter cake production per litre of ethanol produced/kg $litre^{-1}$	3.05	0.85
11. Input/output energy ratio per litre of ethanol produced	11	13

Water is a significant determinant for increased food production and rural development, and for a country like Nigeria that is listed by the FAO (Food and Agriculture Organisation) as being among nations that are technically incapable of achieving food self-sufficiency through rain-fed farming, it may not be economically feasible to rely on sugarcane as a feedstock source for bioethanol production. This is particularly pertinent considering the fact that only about a million hectares of Nigerian cultivable land is currently under irrigation. Furthermore, there is concern that focusing on sugarcane cultivation under irrigation may pose a threat to rice and wheat crop farming in Nigeria.[96]

Conclusion

The choice of bioenergy crop for the production of biofuels is usually determined by geopolitical, economic, social and environmental factors or policy considerations. Importantly, it is quintessential that the bioenergy crop does not overbear on food supplies or the feedstock sources do not lead to significant environmental damage, and the feedstock crops are substantially produced from existing cultivated lands rather than clearing wild virgin lands for cultivation.

Although both sugarcane and sweet sorghum crops are sustainable feedstocks for bioethanol production in Nigeria, sweet sorghum crop is highly productive and inexpensive to grow with good processing characteristics and yielding additional valuable products (grain and fodder), as well as playing a pivotal role in the second-generation or cellulosic bioethanol technology. Comparative analysis of energy cost and yield, especially based on break-even price yield and environmental sustainability, suggests the margin of profit to be in favour of bioethanol production from sweet sorghum relative to sugarcane. Thus, it is envisaged that cultivation of sweet sorghum on industrial scale for bioethanol production will not lead to agrochemical resource consumption competition between energy crops and food crops, while the use of sugarcane as energy crop in Nigeria is envisioned to lead to competition for agrochemicals consumption with food crops.

Interestingly, several local species and varieties of sweet sorghum exist throughout the Savannah–Sahelian region,[30,82-86] including Nigeria. Therefore, the possibilities and potentialities exist to identity and characterize the sweet sorghum varieties most suitable for cultivation and utilization to produce both food and alternative renewable bioenergy. Detailed molecular, biochemical and biophysical studies on local Nigerian sweet sorghum that focus on enzymology, metabolomics, genomics, transcriptomics, and proteomics are needed to optimize fermentable sugar content and bioethanol production.[65,85,87-91] More investment into research and development, as well as enhanced capacity building, is needed to lay sound and effective science and biotechnology policies capable of transforming Nigeria into a major bioenergy producing and exporting country. It is feasible that sustainable development and utilization of local sweet sorghum varieties for biofuels can help to sustain the enviable position of Nigeria as one of the major global fuel producing and exporting countries, even beyond the defunct of current fossil fuel reserves.

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